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Eighth day of July 2002

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AUSTRALIA

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PROVISIONAL SPECIFICATION

Invention Title:

Cytomegalovirus T cell epitopes

The invention is described in the following statement:

Cytomegalovirus T cell Epitopes

FIELD OF THE INVENTION

The present invention relates to compositions for use in raising T cell responses against cytomegalovirus (CMV). In addition the present invention relates to T cell epitopes from CMV, in particular CTL epitopes, and to vaccines for use in protection against and treatment of CMV infection.

BACKGROUND OF THE INVENTION

Human cytomegalovirus (HCMV) is a classic example of a group of herpes viruses which are found universally throughout all geographic locations and socioeconomic groups, and infects between 50 and 85% of adults (Alford and Britt, 1990). For most healthy persons who acquire primary HCMV infection after birth, there are few symptoms and no long-term health consequences. Occasionally, some adults with primary HCMV infection display symptoms of a mononucleosis-like syndrome with prolonged fever, and a mild hepatitis. Once infected with HCMV, the virus remains dormant by establishing a reservoir of latently-infected cells from which chronic low-grade reactivation into the virus productive (lytic) cycle occurs throughout life. Although the factors controlling latency and reactivation are not completely understood, impairment of the body's cell-mediated immune system either by drug-induced immunosuppression or infection by certain pathogens can consistently reactivate the virus (Zaia and Forman, 1995).

HCMV infection is important to certain high-risk groups. Major areas of concern are: (1) the risk of infection to the unborn baby during pregnancy, (2) the risk of infection to people who work with children, and (3) the risk of infection to immunocompromised persons (e.g. organ transplant patients) (Britt, 1996; Plotkin,1999). It is important to mention here that the risk to the foetus appears to be almost exclusively associated with non-immune women who become infected during pregnancy (Fowler et. al., 1992; Murph et. al. 1998). Epidemiological studies have shown that 80%-90% of developing unborn babies who acquire congenital HCMV infection display a variable pattern of pathological sequelae within the first few years of life that may include hearing loss, vision impairment and mental retardation. Another 5%

to 10% of infants who are infected but without symptoms at birth will subsequently develop varying degrees of hearing and mental or coordination problems. In 1996 alone, more than 17,000 cases of HCMV-induced sequelae or death were estimated in Europe and the USA (Plotkin, 1999). In addition, recent studies suggest that HCMV seropositive individuals who have undergone coronary angioplasty develop restenosis more frequently than seronegative patients (Field, 1999), although a causal relationship has yet to be shown. Thus there is a range of clinical situations where HCMV is a significant cause of morbidity and mortality. There is an increasing argument that a reduction in HCMV load in these individuals can provide a significant therapeutic benefit. Vaccination provides the most practical modality of achieving such a reduction in HCMV load.

There have been a number of attempts at designing a vaccine to HCMV (for review see Britt, 1996; Plotkin, 1999). The first were based on immunisation with attenuated strains of HCMV (TOWNE and AD-169) (Elek and Stern, 1974; Neff et. al., 1979). Although both elicited cellular and humoral responses, neither vaccine prevented foetal infection from women experiencing a primary HCMV infection. Furthermore, vaccinated normal volunteers showed limited protection from viral challenge (Toledo strain) (Quinnan et. al., 1984; Adler et. al., 1998). The other approach is based on single HCMV antigen formulations. These include glycoprotein B (gB) with MF59 adjuvant (Chiron) or gB expressed in a viral vector (Pass et. al., 1999; Adler *et. al.* 1999). A canarypox pp65 recombinant has recently been tested in a clinical trial and shown to elicit a strong CTL and antibody response to this antigen (Gyulai et. al., 1999). It should be pointed out that both gB and pp65-based vaccines assume that protection from HCMV can be achieved by induction of immunity against a single antigen which in either case is derived from the virion. There is increasing evidence that in healthy virus carriers, subdominant T cell responses are also directed against other antigens (pp150, IE-1 and gH) which may also play a crucial role in controlling HCMV reactivation (for review see Britt, 1996; Ito, 1998). Indeed, in a recent review Plotkin proposed that the HCMV vaccine should combine in one single regimen all those antigens that might provide protection (Plotkin, 1999).

To develop such a vaccine, viral antigens that activate a protective CTL response need to be identified.

SUMMARY OF THE INVENTION

The present inventors have determined a number of T-cell epitopes, in particular CTL epitopes, from HCMV. A number of these CTL epitopes are derived from HCMV antigens which have not been previously shown or suggested to contain CTL epitopes.

Accordingly in a first aspect the present invention consists in a HCMV T-cell epitope, the epitope having a sequence or being contained within a sequence selected from the group consisting of QMWQARLTV, ALFFFDIDL, LMNGQQIFL, RIFAELEGV, VIGDQYVKV, SLLSEFCRV, VLAELVKQI, ILGADPLRV, TMYGGISLL, LLSEFCRVL, VLEETSVML, CLQNALDIL, ILDEERDKV, GQTEPIAFV, KMSVRETLV, FLGARSPSL, ALVNAVNKL, ALVNFLRHL, NILQKIEKI, LIEDFDIYV, PLIPTTAVI, RIEENLEGV, RIWCLVVCV, QMLLALARL, GLDDLMSGL, IILVAIAVV, DLDEGIMVV, NLFPYLVSA, AVGGAVASV, YINRALAQI, FLMEHTMPV, LMQKFPKQV, NLALSTPFL, IIYTRNHEV, LLGALNLCL, YLMDELRYV, YLTVFTVYL, TLTEDFFVV, LLMMSVYAL, YLLYRMLKT, ILFDGHDLL, LIFGHLPRV, SLVRLVYIL, LLYPTAVDL, ALDPYNEVV, LMLLKNGTV, SAIIGIYLL, QLLIPKSFTL, TLVIPSWHV, LLIPKSFTL, DLVPLTVSV, GLLGASMDL, PLREYLADL, FLLSHDAAL, LLLIVTPVV, LLVEPCARV, LLIDPTSGL, QLRSVIRAL, LLNCAVTKL, TLLVLFIVYV, LLVLFIVYV, SMMWMRFFV, LLFRTLLVYL, YLFSLVVLV, TLLVYLFSL, TMWCLTLFV, SQEPMSIYVY, ATVQGQNLKY, IRETVELRQY, IGDQYVKVY, TVQGQNLKY, YRIQGKLEY, QVIGDQYVK, LLLQRGPQY, RVTGGGAMA, GVMTRGRLK, VYALPLKML, QYDPVAALF, VYYTSAFVF, DIYRIFAEL, DVPSGKLFM, DIDLLLQRG, YVKVYLESF, TVQGQNLKY, EPMSIYVYAL, HVRVSQPSL, QARLTVSGL, RRRHRQDAL, QPKRRRHRQ, LCPKSIPGL, VLCPKNMII, YRIQGKLEY, SEHPTFTSQY, CEDVPSGKLF, NEIHNPAVF, RETVELRQY, QEPMSIYVY, IKEHMLKKY, DEEEAIVAY, CVETMCNEY, KLGGALQAK, QYILGADPL, KYTQTEEKF, KARAKKDEL, VMKRRIEEI, RHRIKEHML, ELRRKMMYM, QIKVRVDMV, NVRRSWEEL, WPRERAWAL, KARDHLAVL, SPWAPTAPL, RPSTPRAAV, VKESPGRCY, LDEGIMVVY, ATSTGDVVY, NTDFRVLEL, AYIYTTYLL, SYENKTMQL, AYEYVDYLF, CYSRPVVIF, KMTATFLSK, IMREFNSYK, KPEPDFTIQY, IIYTRNHEVK, IMKDKNTPF, PRKKKSKRI, ITSLVRLVY, HHEYLSDLY, AIIGIYLLY, QTEKHELLV, ATDSRLLMM, FLDAALDFNY, DTQGVINIMY, LRENTTQCTY, SAIIGIYLLY, SLRNSTVVR, ALALFAAAR, QLNRHSYLK, RLFPDATVP, RLNTYALVSK, LVRLVYILSK,

YLMDELRYVK, ELYLMGSLVH, ALTVSEHVSY, NYLDLSALL, SYVVTNQYL, SYLKDSDFL, TYALVSKDL, SYRSFSQQL, TYGRPIRFL, YYVFHMPRCL, MYMHDSDDVL, ETFPDLFCL, DLTETLERY, SPRTHYLML, FPDLFCLPL, SPRTHYLMLL, MPRCLFAGPL, TPMLLIFGHL, APYQRDNFIL, GRCQMLDRR, RRDHSLERL, SEALDPHAF, RENTTQCTY, DDVLFALDPY, CSDPNTYIHK, EYIVQIQNAF, AEVVARHNPY, LVEPCARVY, GIKHEGLVK, ELLAGGRVF, RLLDLAPNY, ELLGRLNVY, CRYKYLRKK, ARVYEIKCR, VTEHDTLLY, RGDPFDKNY, GLDRNSGNY, TLLNCAVTK, TVRSHCVSK, TRVKRNVKK, YEQHKITSY, SEDSVTFEF, RSYAYIYTTYLLGSNTEYVA, TYEKYGNVSVFETSGGLVVF, FETSGGLVVFWQGIKQKSLV, YHRDSYENKTMQLIPDDYSN, MQLIPDDYSNTHSTRYVTVK, LDEGIMVVYKRNIVAHTFKV, RQTEKHELLVLVKKAQLNRH, ALTVSEHVSYVVTNQYLIKG, CSPDEIMAYAQKIFKILDEE, EEAIVAYTLATAGVSSSDSL, SEPVSEIEEVAPEEEEDGAE, VLCCYVLEETSVMLAKRPLI, TAAKAYAVGQFEQPTETPPE, FEQPTETPPEDLDTLSLAIE, MLPLIKQEDIKPEPDFTIQY, THQLCPRSSDYRNMIIHAAT, YRNMIIHAATPVDLLGALNL, TGPRKKKSKRISELDNEKVR, PVDLLGALNLCLPLMQKFPK, IQIIYTRNHEVKSEVDAVRC, VKSEVDAVRCRLGTMCNLAL, WPRERAWALKNPHLAYNPFR, QQLKAWEERQQNLQQRQQQP, SRDAADEVWALRDQTAESPV, VKPLDLNLDRHANTALVNAV, STSQKPVLGKRVATPHASAR, HANTALVNAVNKLVYTGRLI, LNIPSINVHHYPSAAERKHR, ATVQGQNLKYQEFFWDANDI, QEFFWDANDIYRIFAELEGV, PQYSEHPTFTSQYRIQGKLE, SQYRIQGKLEYRHTWDRHDE, VFTWPPWQAGILARNLVPMV, ILARNLVPMVATVQGQNLKY, DQYVKVYLESFCEDVPSGKL, YPSAAERKHRHLPVADAVIH, QYDPVAALFFFDIDLLLQRG, IIKPGKISHIMLDVAFTSHE, AHELVCSMENTRATKMQVIG, TRATKMQVIGDQYVKVYLES, MNGQQIFLEVQAIRETVELR, QAIRETVELRQYDPVAALFF, LTVSGLAWTRQQNQWKEPDV, WQPAAQPKRRRHRQDALPGP, YRHTWDRHDEGAAQGDDDVW, TSAGRKRKSASSATACTSGV, HRQDALPGPCIASTPKKHRG, YYTSAFVFPTKDVALRHVVC , VTTERKTPRVTGGGAMAGAS, QPFMRPHERNGFTVLCPKNM, SICPSQEPMSIYVYALPLKM, IYVYALPLKMLNIPSINVHH and YYTSAFVFPTKDVALRHVVC.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In a second aspect the present invention consists in a synthetic or recombinant polypeptide, the polypeptide comprising at least two HCMV T-cell epitopes, each epitope having a sequence or being contained within a sequence selected from the group consisting of QMWQARLTV, ALFFFDIDL, LMNGQQIFL, RIFAELEGV, VIGDQYVKV, SLLSEFCRV, VLAELVKQI, ILGADPLRV, TMYGGISLL, LLSEFCRVL, VLEETSVML, CLQNALDIL, ILDEERDKV, GQTEPIAFV, KMSVRETLV, FLGARSPSL, ALVNAVNKL, ALVNFLRHL, NILQKIEKI, LIEDFDIYV, PLIPTTAVI, RIEENLEGV, RIWCLVVCV, QMLLALARL, GLDDLMSGL, IILVAIAVV, DLDEGIMVV, NLFPYLVSA, AVGGAVASV, YINRALAQI, FLMEHTMPV, LMQKFPKQV, NLALSTPFL, IIYTRNHEV, LLGALNLCL, YLMDELRYV, YLTVFTVYL, TLTEDFFVV, LLMMSVYAL, YLLYRMLKT, ILFDGHDLL, LIFGHLPRV, SLVRLVYIL, LLYPTAVDL, ALDPYNEVV, LMLLKNGTV, SAIIGIYLL, QLLIPKSFTL, TLVIPSWHV, LLIPKSFTL, DLVPLTVSV, GLLGASMDL, PLREYLADL, FLLSHDAAL, LLLIVTPVV, LLVEPCARV, LLIDPTSGL, QLRSVIRAL, LLNCAVTKL, TLLVLFIVYV, LLVLFIVYV, SMMWMRFFV, LLFRTLLVYL, YLFSLVVLV, TLLVYLFSL, TMWCLTLFV, SQEPMSIYVY, ATVQGQNLKY, IRETVELRQY, IGDQYVKVY, TVQGQNLKY, YRIQGKLEY, QVIGDQYVK, LLLQRGPQY, RVTGGGAMA, GVMTRGRLK, VYALPLKML, QYDPVAALF, VYYTSAFVF, DIYRIFAEL, DVPSGKLFM, DIDLLLQRG, YVKVYLESF, TVQGQNLKY, EPMSIYVYAL, HVRVSQPSL, QARLTVSGL, RRRHRQDAL, QPKRRRHRQ, LCPKSIPGL, VLCPKNMII, YRIQGKLEY, SEHPTFTSQY, CEDVPSGKLF, NEIHNPAVF, RETVELRQY, QEPMSIYVY, IKEHMLKKY, DEEEAIVAY, CVETMCNEY, KLGGALQAK, QYILGADPL, KYTQTEEKF, KARAKKDEL, VMKRRIEEI, RHRIKEHML, ELRRKMMYM, QIKVRVDMV, NVRRSWEEL, WPRERAWAL, KARDHLAVL, SPWAPTAPL, RPSTPRAAV, VKESPGRCY, LDEGIMVVY, ATSTGDVVY, NTDFRVLEL, AYIYTTYLL, SYENKTMQL, AYEYVDYLF, CYSRPVVIF, KMTATFLSK, IMREFNSYK, KPEPDFTIQY, IIYTRNHEVK, IMKDKNTPF, PRKKKSKRI, ITSLVRLVY, HHEYLSDLY, AIIGIYLLY, QTEKHELLV, ATDSRLLMM, FLDAALDFNY, DTQGVINIMY, LRENITTQCTY, SAIIGIYLLY, SLRNSTVVR, ALALFAAAR, QLNRHSYLK, RLFPDATVP, RLNTYALVSK, LVRLVYILSK, YLMDELRYVK, ELYLMGSLVH, ALTVSEHVSY, NYLDLSALL, SYVVTNQYL, SYLKDSDFL, TYALVSKDL, SYRSFSQQL, TYGRPIRFL, YYVFHMPRCL, MYMHDSDDVL, ETFPDLFCL, DLTETLERY, SPRTHYLML, FPDLFCLPL, SPRTHYLMLL, MPRCLFAGPL, TPMLLIFGHL, APYQRDNFIL, GRCQMLDRR, RRDHSLERL, SEALDPHAF, RENITTQCTY, DDVLFALDPY, CSDPNTYIHK,

EYIVQIQNAF, AEVVARHNPY, LVEPCARVY, GIKHEGLVK, ELLAGGRVF, RLLDLAPNY, ELLGRLNVY, CRYKYLRKK, ARVYEIKCR, VTEHDTLLY, RGDPFDKNY, GLDRNSGNY, TLLNCAVTK, TVRSHCVSK, TRVKRNVKK, YEQHKITSY, SEDSVTFEF, RSYAYIYTTYLLGSNTEYVA, TYEKYGNVSVFETSGGLVVF, FETSGGLVVFWQGIKQKSLV, YHRDSYENKTMQLIPDDYSN, MQLIPDDYSNTHSTRYVTVK, LDEGIMVVYKRNIVAHTFKV, RQTEKHELLVLVKKAQLNRH, ALTVSEHVSYVVTNQYLIKG, CSPDEIMAYAQKIFKILDEE, EEAIVAYTLATAGVSSSDSL, SEPVSEIEEVAPEEEEDGAE, VLCCYVLEETSVMLAKRPLI, TAAKAYAVGQFEQPTETPPE, FEQPTETPPEDLDTLSLAIE, MLPLIKQEDIKPEPDFTIQY, THQLCPRSSDYRNMIIHAAT, YRNMIIHAATPVDLLGALNL, TGPRKKKSKRISELDNEKVR, PVDLLGALNLCLPLMQKFPK, IQIIYTRNHEVKSEVDAVRC, VKSEVDAVRCRLGTMCNLAL, WPRERAWALKNPHLAYNPFR, QQLKAWEERQQNLQQRQQQP, SRDAADEVWALRDQTAESPV, VKPLDLNLDRHANTALVNAV, STSQKPVLGKRVATPHASAR, HANTALVNAVNKLVYTGRLI, LNIPSINVHHYPSAAERKHR, ATVQGQNLKYQEFFWDANDI, QEFFWDANDIYRIFAELEGV, PQYSEHPTFTSQYRIQGKLE, SQYRIQGKLEYRHTWDRHDE, VFTWPPWQAGILARNLVPMV, ILARNLVPMVATVQGQNLKY, DQYVKVYLESFCEDVPSGKL, YPSAAERKHRHLPVADAVIH, QYDPVAALFFFDIDLLLQRG, IIKPGKISHIMLDVAFTSHE, AHELVCSMENTRATKMQVIG, TRATKMQVIGDQYVKVYLES, MNGQQIFLEVQAIRETVELR, QAIRETVELRQYDPVAALFF, LTVSGLAWTRQQNQWKEPDV, WQPAAQPKRRRHRQDALPGP, YRHTWDRHDEGAAQGDDDVW, TSAGRKRKSASSATACTSGV, HRQDALPGPCIASTPKKHRG, YYTSAFVFPTKDVALRHVVC , VTTERKTPRVTGGGAMAGAS, QPFMRPHERNGFTVLCPKNM, SICPSQEPMSIYVYALPLKM, IYVYALPLKMLNIPSINVHH and YYTSAFVFPTKDVALRHVVC.

In a preferred embodiment of the present invention the T-cell epitopes are CTL epitopes.

In a third aspect the present invention consists in a composition for use in raising a T-cell response, in particular a CTL response, against HCMV, the composition comprising a pharmaceutically acceptable carrier and at least one HCMV T-cell epitope of the first aspect of the present invention or the

recombinant or synthetic polypeptide of the second aspect of the present invention.

In a fourth aspect the present invention consists in a composition for use in raising a T-cell response, in particular a CTL response, against HCMV, the composition comprising a pharmaceutically acceptable carrier and DNA encoding at least one HCMV T-cell epitope, the epitope having a sequence or being contained within a sequence selected from the group consisting of QMWQARLTV, ALFFFDIDL, LMNGQQIFL, RIFAELEGV, VIGDQYVKV, SLLSEFCRV, VLAELVKQI, ILGADPLRV, TMYGGISLL, LLSEFCRVL, VLEETSVML, CLQNALDIL, ILDEERDKV, GQTEPIAFV, KMSVRETLV, FLGARSPSL, ALVNAVNKL, ALVNFLRHL, NILQKIEKI, LIEDFDIYV, PLIPTTAVI, RIEENLEGV, RIWCLVVCV, QMLLALARL, GLDDLMSGL, IILVAIAVV, DLDEGIMVV, NLFPYLVSA, AVGGAVASV, YINRALAQI, FLMEHTMPV, LMQKFPKQV, NLALSTPFL, IIYTRNHEV, LLGALNLCL, YLMDELRYV, YLTVFTVYL, TLTEDFFVV, LLMMSVYAL, YLLYRMLKT, ILFDGHDLL, LIFGHLPRV, SLVRLVYIL, LLYPTAVDL, ALDPYNEVV, LMLLKNGTV, SAIIGIYLL, QLLIPKSFTL, TLVIPSWHV, LLIPKSFTL, DLVPLTVSV, GLLGASMDL, PLREYLADL, FLLSHDAAL, LLLIVTPVV, LLVEPCARV, LLIDPTSGL, QLRSVIRAL, LLNCAVTKL, TLLVLFIVYV, LLVLFIVYV, SMMWMRFFV, LLFRTLLVYL, YLFSLVVLV, TLLVYLFSL, TMWCLTLFV, SQEPMSIYVY, ATVQGQNLKY, IRETVELRQY, IGDQYVKVY, TVQGQNLKY, YRIQGKLEY, QVIGDQYVK, LLLQRGPQY, RVTGGGAMA, GVMTRGRLK, VYALPLKML, QYDPVAALF, VYYTSAFVF, DIYRIFAEL, DVPSGKLFM, DIDLLLQRG, YVKVYLESF, TVQGQNLKY, EPMSIYVYAL, HVRVSQPSL, QARLTVSGL, RRRHRQDAL, QPKRRRHRQ, LCPKSIPGL, VLCPKNMII, YRIQGKLEY, SEHPTFTSQY, CEDVPSGKLF, NEIHNPAVF, RETVELRQY, QEPMSIYVY, IKEHMLKKY, DEEEAIVAY, CVETMCNEY, KLGGALQAK, QYILGADPL, KYTQTEEKF, KARAKKDEL, VMKRRIEEI, RHRIKEHML, ELRRKMMYM, QIKVRVDMV, NVRRSWEEL, WPRERAWAL, KARDHLAVL, SPWAPTAPL, RPSTPRAAV, VKESPGRCY, LDEGIMVVY, ATSTGDVVY, NTDFRVLEL, AYIYTTYLL, SYENKTMQL, AYEYVDYLF, CYSRPVVIF, KMTATFLSK, IMREFNSYK, KPEPDFTIQY, IIYTRNHEVK, IMKDKNTPF, PRKKKSKRI, ITSLVRLVY, HHEYLSDLY, AIIGIYLLY, QTEKHELLV, ATDSRLLMM, FLDAALDFNY, DTQGVINIMY, LRENTTQCTY, SAIIGIYLLY, SLRNSTVVR, ALALFAAAR, QLNRHSYLK, RLFPDATVP, RLNTYALVSK, LVRLVYILSK, YLMDELRYVK, ELYLMGSLVH, ALTVSEHVSY, NYLDLSALL, SYVVTNQYL, SYLKDSDFL, TYALVSKDL,

SYRSFSQQL, TYGRPIRFL, YYVFHMPRCL, MYMHDSDDVL, ETFPDLFCL, DLTETLERY, SPRTHYLML, FPDLFCLPL, SPRTHYLMLL, MPRCLFAGPL, TPMLLIFGHL, APYQRDNFIL, GRCQMLDRR, RRDHSLERL, SEALDPHAF, RENTTQCTY, DDVLFALDPY, CSDPNTYIHK, EYIVQIQNAF, AEVVARHNPY, LVEPCARVY, GIKHEGLVK, ELLAGGRVF, RLLDLAPNY, ELLGRLNVY, CRYKYLRKK, ARVYEIKCR, VTEHDTLLY, RGDPFDKNY, GLDRNSGNY, TLLNCAVTK, TVRSHCVSK, TRVKRNVKK, YEQHKITSY, SEDSVTFEF, RSYAYIYTTYLLGSNTEYVA, TYEKYGNVSVFETSGGLVVF, FETSGGLVVFWQGIKQKSLV, YHRDSYENKTMQLIPDDYSN, MQLIPDDYSNTHSTRYVTVK, LDEGIMVVYKRNIVAHTFKV, RQTEKHELLVLVKKAQLNRH, ALTVSEHVSYVVTNQYLIKG, CSPDEIMAYAQKIFKILDEE, EEAIVAYTLATAGVSSSDSL, SEPVSEIEEVAPEEEEDGAE, VLCCYVLEETSVMLAKRPLI, TAAKAYAVGQFEQPTETPPE, FEQPTETPPEDLDTLSLAIE, MLPLIKQEDIKPEPDFTIQY, THQLCPRSSDYRNMIIHAAT, YRNMIIHAATPVDLLGALNL, TGPRKKKSKRISELDNEKVR, PVDLLGALNLCLPLMQKFPK, IQIIYTRNHEVKSEVDAVRC, VKSEVDAVRCRLGTMCNLAL, WPRERAWALKNPHLAYNPFR, QQLKAWEERQQNLQQRQQQP, SRDAADEVWALRDQTAESPV, VKPLDLNLDRHANTALVNAV, STSQKPVLGKRVATPHASAR, HANTALVNAVNKLVYTGRLI, LNIPSINVHHYPSAAERKHR, ATVQGQNLKYQEFFWDANDI, QEFFWDANDIYRIFAELEGV, PQYSEHPTFTSQYRIQGKLE, SQYRIQGKLEYRHTWDRHDE, VFTWPPWQAGILARNLVPMV, ILARNLVPMVATVQGQNLKY, DQYVKVYLESFCEDVPSGKL, YPSAAERKHRHLPVADAVIH, QYDPVAALFFFDIDLLLQRG, IIKPGKISHIMLDVAFTSHE, AHELVCSMENTRATKMQVIG, TRATKMQVIGDQYVKVYLES, MNGQQIFLEVQAIRETVELR, QAIRETVELRQYDPVAALFF, LTVSGLAWTRQQNQWKEPDV, WQPAAQPKRRRHRQDALPGP, YRHTWDRHDEGAAQGDDDVW, TSAGRKRKSASSATACTSGV, HRQDALPGPCIASTPKKHRG, YYTSAFVFPTKDVALRHVVC , VTTERKTPRVTGGGAMAGAS, QPFMRPHERNGFTVLCPKNM, SICPSQEPMSIYVYALPLKM, IYVYALPLKMLNIPSINVHH and YYTSAFVFPTKDVALRHVVC.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In a fifth aspect the present invention consists in a method of reducing the risk of treating HCMV infection in a subject, the method comprising administering to the subject the composition of the third or fourth aspect of the present invention.

BRIEF DECSRIPTION OF FIGURES

Figure 1: MHC stabilization on T2 cells using potential HLA A2-binding peptides from HCMV antigens (pp65, IE-1, gH, pp71, gB, IE-2, pp150, pp50, pp28, US3, US2, UL18). T2 cells were initially incubated with 100µl of each of the peptide (200µg/ml) for 14-16 h at 26°C, followed by incubation at 37°C for 2-3 h. HLA A2 expresion on these cells was analysed by flow cytometery using BB7.2 antibody. The dotted line indicates the mean+ 3SD of the fluorescence intensity for HLA A2 on T2 cells incubated at 26°C without peptide.

Figure 2: *Ex vivo* functional analysis of HCMV-specific CTL responses in a panel of eight HLA A2-positive healthy seropositive individuals. Potential peptide epitopes from ten different antigens of HCMV, pp65 (panel A), IE-1 (panel B), pp150 (panel C), pp28 (panel D), US2 (panel E), US3 (panel F), pp50 (panel G), gB (panel H), gH (panel I) and IE-2 (panel J) were tested using ELISPOT assays. PBMC from healthy seropositve individuals were stimulated with individual synthetic peptides from these antigens and IFN-γ production was measured in ELISPOT assays as described in the Material and Methods section. The results are expressed as spot forming cells (SFC) per 10^6 PBMC.

Figure 3: Recognition of HCMV peptide epitopes by polyclonal CTLs from HLA A2-positive donor SB. PBMC from donor SB were co-cultivated with peptide sensitised (20µg/ml) autologous PBMC at a ratio of 2:1 for 7 days. On day 7 these cultures were restimulated with aulogous γ-irradiated EBV-transformed LCLs sensitised with peptide epitopes. On day 10 these T cell lines were used as polyclonal effectors in a standard ^{51}Cr-release assay against peptide sensitized autologous PHA blasts. An effector:target ratio of 10:1 was used these assays. Results are expressed as percent specific lysis.

Figure 4: Analysis of HCMV peptide epitopes using virus-specific CTL clones from healthy virus carriers. CTL clones from healthy virus carriers were isolated as described in the "Material and Methods" section.

Representative data from three different CTL clones specific for peptide epitopes VLEETSVML (panel A; IE-1), NLVPMVATV (panel B; pp65) and IIYTRNHEV (panel C; IE-2) are shown. CTL clones specific for each of these peptide epitope were tested against autologous PHA blast presensitised with varying concentrations of synthetic peptides (shown on x-axis). Results are expressed as percent specific lysis.

Figure 5: MHC stabilization on T2 cells using potential HLA B7 binding peptides from HCMV antigens (pp65, IE-1, gH and pp150). T2 cells were initially inclubated with 100µl of each of the peptide (200µg/ml) for 14-16 h at 26°C, followed by incubation at 37°C for 2-3 h. HLA A2 expresion on these cells was analysed by flow cytometery using BB7.1 antibody. The dotted line indicates the mean+ 3SD of the fluorescence intensity for HLA B7 on T2 cells incubated at 26°C without peptide.

Figure 6: Ex vivo functional analysis of HCMV-specific CTL responses against HLA B8, HLA B7, HLA A24, HLA B27, B44, A3 and HLA A1-restricted potential CTL epitopes. Peptide epitopes from HCMV antigens, pp65, IE-1, pp150, gH, pp28 and pp50 were tested using ELISPOT assays. PBMC from healthy seropositve individuals were stimulated with individual synthetic peptides from these antigens and IFN-γ production was measured in ELISPOT assay as described in the "Material and Methods" section. The results are expressed as spot forming cells (SFC) per 10^6 PBMC.

DETAILED DESCRIPTION

In a first aspect the present invention consists in a HCMV T-cell epitope, the epitope having a sequence or being contained within a sequence selected from the group consisting of QMWQARLTV, ALFFFDIDL, LMNGQQIFL, RIFAELEGV, VIGDQYVKV, SLLSEFCRV, VLAELVKQI, ILGADPLRV, TMYGGISLL, LLSEFCRVL, VLEETSVML, CLQNALDIL, ILDEERDKV, GQTEPIAFV, KMSVRETLV, FLGARSPSL, ALVNAVNKL, ALVNFLRHL, NILQKIEKI, LIEDFDIYV, PLIPTTAVI, RIEENLEGV, RIWCLVVCV, QMLLALARL, GLDDLMSGL, IILVAIAVV, DLDEGIMVV, NLFPYLVSA, AVGGAVASV, YINRALAQI, FLMEHTMPV, LMQKFPKQV, NLALSTPFL, IIYTRNHEV, LLGALNLCL, YLMDELRYV, YLTVFTVYL, TLTEDFFVV, LLMMSVYAL, YLLYRMLKT, ILFDGHDLL, LIFGHLPRV, SLVRLVYIL, LLYPTAVDL, ALDPYNEVV, LMLLKNGTV, SAIIGIYLL,

QLLIPKSFTL, TLVIPSWHV, LLIPKSFTL, DLVPLTVSV, GLLGASMDL, PLREYLADL, FLLSHDAAL, LLLIVTPVV, LLVEPCARV, LLIDPTSGL, QLRSVIRAL, LLNCAVTKL, TLLVLFIVYV, LLVLFIVYV, SMMWMRFFV, LLFRTLLVYL, YLFSLVVLV, TLLVYLFSL, TMWCLTLFV, SQEPMSIYVY, ATVQGQNLKY, IRETVELRQY, IGDQYVKVY, TVQGQNLKY, YRIQGKLEY, QVIGDQYVK, LLLQRGPQY, RVTGGGAMA, GVMTRGRLK, VYALPLKML, QYDPVAALF, VYYTSAFVF, DIYRIFAEL, DVPSGKLFM, DIDLLLQRG, YVKVYLESF, TVQGQNLKY, EPMSIYVYAL, HVRVSQPSL, QARLTVSGL, RRRHRQDAL, QPKRRRHRQ, LCPKSIPGL, VLCPKNMII, YRIQGKLEY, SEHPTFTSQY, CEDVPSGKLF, NEIHNPAVF, RETVELRQY, QEPMSIYVY, IKEHMLKKY, DEEEAIVAY, CVETMCNEY, KLGGALQAK, QYILGADPL, KYTQTEEKF, KARAKKDEL, VMKRRIEEI, RHRIKEHML, ELRRKMMYM, QIKVRVDMV, NVRRSWEEL, WPRERAWAL, KARDHLAVL, SPWAPTAPL, RPSTPRAAV, VKESPGRCY, LDEGIMVVY, ATSTGDVVY, NTDFRVLEL, AYIYTTYLL, SYENKTMQL, AYEYVDYLF, CYSRPVVIF, KMTATFLSK, IMREFNSYK, KPEPDFTIQY, IIYTRNHEVK, IMKDKNTPF, PRKKKSKRI, ITSLVRLVY, HHEYLSDLY, AIIGIYLLY, QTEKHELLV, ATDSRLLMM, FLDAALDFNY, DTQGVINIMY, LRENTTQCTY, SAIIGIYLLY, SLRNSTVVR, ALALFAAAR, QLNRHSYLK, RLFPDATVP, RLNTYALVSK, LVRLVYILSK, YLMDELRYVK, ELYLMGSLVH, ALTVSEHVSY, NYLDLSALL, SYVVTNQYL, SYLKDSDFL, TYALVSKDL, SYRSFSQQL, TYGRPIRFL, YYVFHMPRCL, MYMHDSDDVL, ETFPDLFCL, DLTETLERY, SPRTHYLML, FPDLFCLPL, SPRTHYLMLL, MPRCLFAGPL, TPMLLIFGHL, APYQRDNFIL, GRCQMLDRR, RRDHSLERL, SEALDPHAF, RENTTQCTY, DDVLFALDPY, CSDPNTYIHK, EYIVQIQNAF, AEVVARHNPY, LVEPCARVY, GIKHEGLVK, ELLAGGRVF, RLLDLAPNY, ELLGRLNVY, CRYKYLRKK, ARVYEIKCR, VTEHDTLLY, RGDPFDKNY, GLDRNSGNY, TLLNCAVTK, TVRSHCVSK, TRVKRNVKK, YEQHKITSY, SEDSVTFEF, RSYAYIYTTYLLGSNTEYVA, TYEKYGNVSVFETSGGLVVF, FETSGGLVVFWQGIKQKSLV, YHRDSYENKTMQLIPDDYSN, MQLIPDDYSNTHSTRYVTVK, LDEGIMVVYKRNIVAHTFKV, RQTEKHELLVLVKKAQLNRH, ALTVSEHVSYVVTNQYLIKG, CSPDEIMAYAQKIFKILDEE, EEAIVAYTLATAGVSSSDSL, SEPVSEIEEVAPEEEEDGAE, VLCCYVLEETSVMLAKRPLI, TAAKAYAVGQFEQPTETPPE, FEQPTETPPEDLDTLSLAIE, MLPLIKQEDIKPEPDFTIQY, THQLCPRSSDYRNMIIHAAT, YRNMIIHAATPVDLLGALNL, TGPRKKKSKRISELDNEKVR, PVDLLGALNLCLPLMQKFPK,

IQIIYTRNHEVKSEVDAVRC, VKSEVDAVRCRLGTMCNLAL, WPRERAWALKNPHLAYNPFR, QQLKAWEERQQNLQQRQQQP, SRDAADEVWALRDQTAESPV, VKPLDLNLDRHANTALVNAV, STSQKPVLGKRVATPHASAR, HANTALVNAVNKLVYTGRLI, LNIPSINVHHYPSAAERKHR, ATVQGQNLKYQEFFWDANDI, QEFFWDANDIYRIFAELEGV, PQYSEHPTFTSQYRIQGKLE, SQYRIQGKLEYRHTWDRHDE, VFTWPPWQAGILARNLVPMV, ILARNLVPMVATVQGQNLKY, DQYVKVYLESFCEDVPSGKL, YPSAAERKHRHLPVADAVIH, QYDPVAALFFFDIDLLLQRG, IIKPGKISHIMLDVAFTSHE, AHELVCSMENTRATKMQVIG, TRATKMQVIGDQYVKVYLES, MNGQQIFLEVQAIRETVELR, QAIRETVELRQYDPVAALFF, LTVSGLAWTRQQNQWKEPDV, WQPAAQPKRRRHRQDALPGP, YRHTWDRHDEGAAQGDDDVW, TSAGRKRKSASSATACTSGV, HRQDALPGPCIASTPKKHRG, YYTSAFVFPTKDVALRHVVC , VTTERKTPRVTGGGAMAGAS, QPFMRPHERNGFTVLCPKNM, SICPSQEPMSIYVYALPLKM, IYVYALPLKMLNIPSINVHH and YYTSAFVFPTKDVALRHVVC.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In a second aspect the present invention consists in a synthetic or recombinant polypeptide, the polypeptide comprising at least two HCMV T-cell epitopes, each epitope having a sequence or being contained within a sequence selected from the group consisting of QMWQARLTV, ALFFFDIDL, LMNGQQIFL, RIFAELEGV, VIGDQYVKV, SLLSEFCRV, VLAELVKQI, ILGADPLRV, TMYGGISLL, LLSEFCRVL, VLEETSVML, CLQNALDIL, ILDEERDKV, GQTEPIAFV, KMSVRETLV, FLGARSPSL, ALVNAVNKL, ALVNFLRHL, NILQKIEKI, LIEDFDIYV, PLIPTTAVI, RIEENLEGV, RIWCLVVCV, QMLLALARL, GLDDLMSGL, IILVAIAVV, DLDEGIMVV, NLFPYLVSA, AVGGAVASV, YINRALAQI, FLMEHTMPV, LMQKFPKQV, NLALSTPFL, IIYTRNHEV, LLGALNLCL, YLMDELRYV, YLTVFTVYL, TLTEDFFVV, LLMMSVYAL, YLLYRMLKT, ILFDGHDLL, LIFGHLPRV, SLVRLVYIL, LLYPTAVDL, ALDPYNEVV, LMLLKNGTV, SAIIGIYLL, QLLIPKSFTL, TLVIPSWHV, LLIPKSFTL, DLVPLTVSV, GLLGASMDL, PLREYLADL, FLLSHDAAL, LLLIVTPVV, LLVEPCARV, LLIDPTSGL, QLRSVIRAL, LLNCAVTKL, TLLVLFIVYV, LLVLFIVYV, SMMWMRFFV, LLFRTLLVYL, YLFSLVVLV, TLLVYLFSL, TMWCLTLFV, SQEPMSIYVY, ATVQGQNLKY, IRETVELRQY, IGDQYVKVY, TVQGQNLKY, YRIQGKLEY,

QVIGDQYVK, LLLQRGPQY, RVTGGGAMA, GVMTRGRLK, VYALPLKML, QYDPVAALF, VYYTSAFVF, DIYRIFAEL, DVPSGKLFM, DIDLLLQRG, YVKVYLESF, TVQGQNLKY, EPMSIYVYAL, HVRVSQPSL, QARLTVSGL, RRRHRQDAL, QPKRRRHRQ, LCPKSIPGL, VLCPKNMII, YRIQGKLEY, SEHPTFTSQY, CEDVPSGKLF, NEIHNPAVF, RETVELRQY, QEPMSIYVY, IKEHMLKKY, DEEEAIVAY, CVETMCNEY, KLGGALQAK, QYILGADPL, KYTQTEEKF, KARAKKDEL, VMKRRIEEI, RHRIKEHML, ELRRKMMYM, QIKVRVDMV, NVRRSWEEL, WPRERAWAL, KARDHLAVL, SPWAPTAPL, RPSTPRAAV, VKESPGRCY, LDEGIMVVY, ATSTGDVVY, NTDFRVLEL, AYIYTTYLL, SYENKTMQL, AYEYVDYLF, CYSRPVVIF, KMTATFLSK, IMREFNSYK, KPEPDFTIQY, IIYTRNHEVK, IMKDKNTPF, PRKKKSKRI, ITSLVRLVY, HHEYLSDLY, AIIGIYLLY, QTEKHELLV, ATDSRLLMM, FLDAALDFNY, DTQGVINIMY, LRENTTQCTY, SAIIGIYLLY, SLRNSTVVR, ALALFAAAR, QLNRHSYLK, RLFPDATVP, RLNTYALVSK, LVRLVYILSK, YLMDELRYVK, ELYLMGSLVH, ALTVSEHVSY, NYLDLSALL, SYVVTNQYL, SYLKDSDFL, TYALVSKDL, SYRSFSQQL, TYGRPIRFL, YYVFHMPRCL, MYMHDSDDVL, ETFPDLFCL, DLTETLERY, SPRTHYLML, FPDLFCLPL, SPRTHYLMLL, MPRCLFAGPL, TPMLLIFGHL, APYQRDNFIL, GRCQMLDRR, RRDHSLERL, SEALDPHAF, RENTTQCTY, DDVLFALDPY, CSDPNTYIHK, EYIVQIQNAF, AEVVARHNPY, LVEPCARVY, GIKHEGLVK, ELLAGGRVF, RLLDLAPNY, ELLGRLNVY, CRYKYLRKK, ARVYEIKCR, VTEHDTLLY, RGDPFDKNY, GLDRNSGNY, TLLNCAVTK, TVRSHCVSK, TRVKRNVKK, YEQHKITSY, SEDSVTFEF, RSYAYIYTTYLLGSNTEYVA, TYEKYGNVSVFETSGGLVVF, FETSGGLVVFWQGIKQKSLV, YHRDSYENKTMQLIPDDYSN, MQLIPDDYSNTHSTRYVTVK, LDEGIMVVYKRNIVAHTFKV, RQTEKHELLVLVKKAQLNRH, ALTVSEHVSYVVTNQYLIKG, CSPDEIMAYAQKIFKILDEE, EEAIVAYTLATAGVSSSDSL, SEPVSEIEEVAPEEEEDGAE, VLCCYVLEETSVMLAKRPLI, TAAKAYAVGQFEQPTETPPE, FEQPTETPPEDLDTLSLAIE, MLPLIKQEDIKPEPDFTIQY, THQLCPRSSDYRNMIIHAAT, YRNMIIHAATPVDLLGALNL, TGPRKKKSKRISELDNEKVR, PVDLLGALNLCLPLMQKFPK, IQIIYTRNHEVKSEVDAVRC, VKSEVDAVRCRLGTMCNLAL, WPRERAWALKNPHLAYNPFR, QQLKAWEERQQNLQQRQQQP, SRDAADEVWALRDQTAESPV, VKPLDLNLDRHANTALVNAV, STSQKPVLGKRVATPHASAR, HANTALVNAVNKLVYTGRLI, LNIPSINVHHYPSAAERKHR, ATVQGQNLKYQEFFWDANDI,

QEFFWDANDIYRIFAELEGV, PQYSEHPTFTSQYRIQGKLE, SQYRIQGKLEYRHTWDRHDE, VFTWPPWQAGILARNLVPMV, ILARNLVPMVATVQGQNLKY, DQYVKVYLESFCEDVPSGKL, YPSAAERKHRHLPVADAVIH, QYDPVAALFFFDIDLLLQRG, IIKPGKISHIMLDVAFTSHE, AHELVCSMENTRATKMQVIG, TRATKMQVIGDQYVKVYLES, MNGQQIFLEVQAIRETVELR, QAIRETVELRQYDPVAALFF, LTVSGLAWTRQQNQWKEPDV, WQPAAQPKRRRHRQDALPGP, YRHTWDRHDEGAAQGDDDVW, TSAGRKRKSASSATACTSGV, HRQDALPGPCIASTPKKHRG, YYTSAFVFPTKDVALRHVVC , VTTERKTPRVTGGGAMAGAS, QPFMRPHERNGFTVLCPKNM, SICPSQEPMSIYVYALPLKM, IYVYALPLKMLNIPSINVHH and YYTSAFVFPTKDVALRHVVC.

In a preferred embodiment of the present invention the T-cell epitopes are CTL epitopes.

In a third aspect the present invention consists in a composition for use in raising a T-cell response, in particular a CTL response, against HCMV, the composition comprising a pharmaceutically acceptable carrier and at least one HCMV T-cell epitope of the first aspect of the present invention or the recombinant or synthetic polypeptide of the second aspect of the present invention.

In a fourth aspect the present invention consists in a composition for use in raising a T-cell response, in particular a CTL response, against HCMV, the composition comprising a pharmaceutically acceptable carrier and DNA encoding at least one HCMV T-cell epitope, the epitope having a sequence or being contained within a sequence selected from the group consisting of QMWQARLTV, ALFFFDIDL, LMNGQQIFL, RIFAELEGV, VIGDQYVKV, SLLSEFCRV, VLAELVKQI, ILGADPLRV, TMYGGISLL, LLSEFCRVL, VLEETSVML, CLQNALDIL, ILDEERDKV, GQTEPIAFV, KMSVRETLV, FLGARSPSL, ALVNAVNKL, ALVNFLRHL, NILQKIEKI, LIEDFDIYV, PLIPTTAVI, RIEENLEGV, RIWCLVVCV, QMLLALARL, GLDDLMSGL, IILVAIAVV, DLDEGIMVV, NLFPYLVSA, AVGGAVASV, YINRALAQI, FLMEHTMPV, LMQKFPKQV, NLALSTPFL, IIYTRNHEV, LLGALNLCL, YLMDELRYV, YLTVFTVYL, TLTEDFFVV, LLMMSVYAL, YLLYRMLKT, ILFDGHDLL, LIFGHLPRV, SLVRLVYIL, LLYPTAVDL, ALDPYNEVV, LMLLKNGTV, SAIIGIYLL, QLLIPKSFTL, TLVIPSWHV, LLIPKSFTL, DLVPLTVSV, GLLGASMDL, PLREYLADL, FLLSHDAAL, LLLIVTPVV, LLVEPCARV, LLIDPTSGL, QLRSVIRAL, LLNCAVTKL, TLLVLFIVYV,

LLVLFIVYV, SMMWMRFFV, LLFRTLLVYL, YLFSLVVLV, TLLVYLFSL,
TMWCLTLFV, SQEPMSIYVY, ATVQGQNLKY, IRETVELRQY, IGDQYVKVY,
TVQGQNLKY, YRIQGKLEY, QVIGDQYVK, LLLQRGPQY, RVTGGGAMA,
GVMTRGRLK, VYALPLKML, QYDPVAALF, VYYTSAFVF, DIYRIFAEL,
DVPSGKLFM, DIDLLLQRG, YVKVYLESF, TVQGQNLKY, EPMSIYVYAL,
HVRVSQPSL, QARLTVSGL, RRRHRQDAL, QPKRRRHRQ, LCPKSIPGL,
VLCPKNMII, YRIQGKLEY, SEHPTFTSQY, CEDVPSGKLF, NEIHNPAVF,
RETVELRQY, QEPMSIYVY, IKEHMLKKY, DEEEAIVAY, CVETMCNEY,
KLGGALQAK, QYILGADPL, KYTQTEEKF, KARAKKDEL, VMKRRIEEI,
RHRIKEHML, ELRRKMMYM, QIKVRVDMV, NVRRSWEEL, WPRERAWAL,
KARDHLAVL, SPWAPTAPL, RPSTPRAAV, VKESPGRCY, LDEGIMVVY,
ATSTGDVVY, NTDFRVLEL, AYIYTTYLL, SYENKTMQL, AYEYVDYLF,
CYSRPVVIF, KMTATFLSK, IMREFNSYK, KPEPDFTIQY, IIYTRNHEVK,
IMKDKNTPF, PRKKKSKRI, ITSLVRLVY, HHEYLSDLY, AIIGIYLLY,
QTEKHELLV, ATDSRLLMM, FLDAALDFNY, DTQGVINIMY, LRENTTQCTY,
SAIIGIYLLY, SLRNSTVVR, ALALFAAAR, QLNRHSYLK, RLFPDATVP,
RLNTYALVSK, LVRLVYILSK, YLMDELRYVK, ELYLMGSLVH,
ALTVSEHVSY, NYLDLSALL, SYVVTNQYL, SYLKDSDFL, TYALVSKDL,
SYRSFSQQL, TYGRPIRFL, YYVFHMPRCL, MYMHDSDDVL, ETFPDLFCL,
DLTETLERY, SPRTHYLML, FPDLFCLPL, SPRTHYLMLL, MPRCLFAGPL,
TPMLLIFGHL, APYQRDNFIL, GRCQMLDRR, RRDHSLERL, SEALDPHAF,
RENTTQCTY, DDVLFALDPY, CSDPNTYIHK, EYIVQIQNAF, AEVVARHNPY,
LVEPCARVY, GIKHEGLVK, ELLAGGRVF, RLLDLAPNY, ELLGRLNVY,
CRYKYLRKK, ARVYEIKCR, VTEHDTLLY, RGDPFDKNY, GLDRNSGNY,
TLLNCAVTK, TVRSHCVSK, TRVKRNVKK, YEQHKITSY, SEDSVTFEF,
RSYAYIYTTYLLGSNTEYVA, TYEKYGNVSVFETSGGLVVF,
FETSGGLVVFWQGIKQKSLV, YHRDSYENKTMQLIPDDYSN,
MQLIPDDYSNTHSTRYVTVK, LDEGIMVVYKRNIVAHTFKV,
RQTEKHELLVLVKKAQLNRH, ALTVSEHVSYVVTNQYLIKG,
CSPDEIMAYAQKIFKILDEE, EEAIVAYTLATAGVSSSDSL,
SEPVSEIEEVAPEEEEDGAE, VLCCYVLEETSVMLAKRPLI,
TAAKAYAVGQFEQPTETPPE, FEQPTETPPEDLDTLSLAIE,
MLPLIKQEDIKPEPDFTIQY, THQLCPRSSDYRNMIIHAAT,
YRNMIIHAATPVDLLGALNL, TGPRKKKSKRISELDNEKVR,
PVDLLGALNLCLPLMQKFPK, IQIIYTRNHEVKSEVDAVRC,
VKSEVDAVRCRLGTMCNLAL, WPRERAWALKNPHLAYNPFR,
QQLKAWEERQQNLQQRQQQP, SRDAADEVWALRDQTAESPV,

VKPLDLNLDRHANTALVNAV, STSQKPVLGKRVATPHASAR, HANTALVNAVNKLVYTGRLI, LNIPSINVHHYPSAAERKHR, ATVQGQNLKYQEFFWDANDI, QEFFWDANDIYRIFAELEGV, PQYSEHPTFTSQYRIQGKLE, SQYRIQGKLEYRHTWDRHDE, VFTWPPWQAGILARNLVPMV, ILARNLVPMVATVQGQNLKY, DQYVKVYLESFCEDVPSGKL, YPSAAERKHRHLPVADAVIH, QYDPVAALFFFDIDLLLQRG, IIKPGKISHIMLDVAFTSHE, AHELVCSMENTRATKMQVIG, TRATKMQVIGDQYVKVYLES, MNGQQIFLEVQAIRETVELR, QAIRETVELRQYDPVAALFF, LTVSGLAWTRQQNQWKEPDV, WQPAAQPKRRRHRQDALPGP, YRHTWDRHDEGAAQGDDDVW, TSAGRKRKSASSATACTSGV, HRQDALPGPCIASTPKKHRG, YYTSAFVFPTKDVALRHVVC , VTTERKTPRVTGGGAMAGAS, QPFMRPHERNGFTVLCPKNM, SICPSQEPMSIYVYALPLKM, IYVYALPLKMLNIPSINVHH and YYTSAFVFPTKDVALRHVVC.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In a fifth aspect the present invention consists in a method of reducing the risk of treating HCMV infection in a subject, the method comprising administering to the subject the composition of the third or fourth aspect of the present invention.

As will be understood by persons skilled in this field substitutions can be made in the peptide sequences set out above. It is intended that such substituted peptides which retain their activity as T-cell epitopes are included within the scope of the present invention.

Typically such substitutions will be so called "conservative" substitutions. It is well recognised that frequently an amino acid having particular physical characteristics, such as charge, size, hydrophobicity etc. can be substituted in peptide with an amino acid having similar physical characteristics and that the modified peptide retains substantially the same biological activity of the unmodified peptide.

While the concept of conservative substitution is well known in the field illustrative substitutions are exemplified below.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and

preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

As is described in PCT/AU95/00461, the disclosure of which is included herein by cross reference, a number of DNA sequences encoding CTL epitopes or CTL peptide epitopes can be combined in a single construct and each epitope be correctly processed. Such a construct is referred to as a "POLYTOPE™". In a preferred embodiment of the second and fourth aspect the CTL epitopes are a POLYTOPE™.

Where the composition comprises the synthetic or recombinant polypeptide of the present invention it is preferred that the composition further comprises ISCOMs® and/or ISCOMATRIX®.

Pharmaceutically acceptable carriers or diluents include those used in compositions suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. They are non-toxic to recipients at the dosages and concentrations employed. Representative examples of pharmaceutically acceptable carriers or diluents include, but are not limited to water, isotonic solutions which are preferably buffered at a physiological pH (such as phosphate-buffered saline or Tris-buffered saline) and can also contain one or more of, mannitol, lactose, trehalose, dextrose, glycerol, ethanol or polypeptides (such as human serum albumin). The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

It is preferred that the composition includes an adjuvant. As will be understood an "adjuvant" means a composition comprised of one or more substances that enhances the immunogenicity and efficacy of a vaccine composition. Non-limiting examples of suitable adjuvants include squalane

and squalene (or other oils of animal origin); block copolymers; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol or Marcol, vegetable oils such as peanut oil; *Corynebacterium*-derived adjuvants such as *Corynebacterium parvum*; *Propionibacterium*-derived adjuvants such as *Propionibacterium acne*; *Mycobacterium bovis* (Bacille Calmette and Guerin or BCG); interleukins such as interleukin 2 and interleukin 12; monokines such as interleukin 1; tumour necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminium hydroxide or Quil-A aluminium hydroxide; liposomes; ISCOM® and ISCOMATRIX® adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as muramyl dipeptides or other derivatives; Avridine; Lipid A derivatives; dextran sulfate; DEAE-Dextran or with aluminium phosphate; carboxypolymethylene such as Carbopol' EMA; acrylic copolymer emulsions such as Neocryl A640 (e.g. U.S. Pat. No. 5,047,238); vaccinia or animal poxvirus proteins; sub-viral particle adjuvants such as cholera toxin, or mixtures thereof.

In a further preferred embodiment the composition of the present invention further comprises at least one HCMV protein or polypeptide which includes at least one HCMV B-cell epitope. It is preferred that the composition further comprises the HCMV antigen gB or fragment thereof comprising at least one B-cell epitope.

The HCMV T-cell epitopes or combination of epitopes may be produced by recombinant DNA technology or using standard chemical synthesis techniques. The decision as to whether recombiant or chemical synthesis techniques are used will typically depend on the length of the polypeptide desired.

Where the epitopes are to be chemically synthesised this may be done as described, for example, in Chapter 9 entitled "Peptide Synthesis" by Atherton and Sheppard which is included in a publication entitled "Synthetic Vaccines" edited by Nicholson and published by Blackwell Scientific Publications. Preferably a solid phase support is utilised which may be polystyrene gel beads wherein the polystyrene may be cross-linked with a small proportion of divinylbenzene (e.g. 1%) which is further swollen by lipophilic solvents such as dichloromethane or more polar solvents such as dimethylformamide (DMF). The polystyrene may be functionalised with chloromethyl or aminomethyl groups. Alternatively, cross-linked and functionalised polydimethyl-acrylamide gel is used which may be highly solvated and swollen by DMF and other dipolar aprotic solvents. Other

supports can be utilised based on polyethylene glycol which is usually grafted or otherwise attached to the surface of inert polystyrene beads. In a preferred form, use may be made of commercial solid supports or resins which are selected from PAL-PEG-PS, PAC-PEG-PS, KA, KR or TGR.

In solid state synthesis, use is made of reversible blocking groups which have the dual function of masking unwanted reactivity in the α-amino, carboxy or side chain functional groups and of destroying the dipolar character of amino acids and peptides which render them inactive. Such functional groups can be selected from t-butyl esters of the structure RCO-$OCMe_3$-CO. Use may also be made of the corresponding benzyl esters having the structure RCO-OCH_2-C_6H_5 and urethanes having the structure $C_6H_5CH_2OCO$-NHR which are known as the benzyloxycarbonyl or Z-derivatives and any Me_3-COCO-NHR, which are known as t-butoxyl carbonyl, or Boc derivatives. Use may also be made of derivatives of fluorenyl methanol and especially the fluorenyl-methoxy carbonyl or Fmoc group. Each of these types of protecting group is capable of independent cleavage in the presence of one other so that frequent use is made, for example, of BOC-benzyl and Fmoc-tertiary butyl protection strategies.

Reference also should be made to a condensing agent to link the amino and carboxy groups of protected amino acids or peptides. This may be done by activating the carboxy group so that it reacts spontaneously with a free primary or secondary amine. Activated esters such as those derived from p-nitrophenol and pentafluorophenol may be used for this purpose. Their reactivity may be increased by addition of catalysts such as 1-hydroxybenzotriazole. Esters of triazine DHBT (as discussed on page 215-216 of the abovementioned Nicholson reference) also may be used. Other acylating species are formed in situ by treatment of the carboxylic acid (i.e. the N-alpha-protected amino acid or peptide) with a condensing reagent and are reacted immediately with the amino component (the carboxy or C-protected amino acid or peptide). Dicyclohexylcarbodiimide, the BOP reagent (referred to on page 216 of the Nicholson reference), O'Benzotriazole-N, N, N'N'-tetra methyl-uronium hexafluorophosphate (HBTU) and its analogous tetrafluoroborate are frequently used condensing agents.

The attachment of the first amino acid to the solid phase support may be carried out using BOC-amino acids in any suitable manner. In one method BOC amino acids are attached to chloromethyl resin by warming the triethyl

ammonium salts with the resin. Fmoc-amino acids may be coupled to the p-alkoxybenzyl alcohol resin in similar manner. Alternatively, use may be made of various linkage agents or "handles" to join the first amino acid to the resin. In this regard, p-hydroxymethyl phenylacetic acid linked to aminomethyl polystyrene may be used for this purpose.

Where it is desired to produce the epitopes recombinantly techniques well known in the art will be employed. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present). The disclosure of these texts are incorporated herein by reference.

In further aspect the present invention consists in a T-cell epitope derived from pp65 of HCMV, the T-cell epitope having a sequence selected from the group consisting of QMWQARLTV, ALFFFDIDL, LMNGQQIFL, RIFAELEGV, VIGDQYVKV, SQEPMSIYVY, ATVQGQNLKY, IRETVELRQY, IGDQYVKVY, TVQGQNLKY, YRIQGKLEY, QVIGDQYVK, LLLQRGPQY, RVTGGGAMA, GVMTRGRLK, VYALPLKML, QYDPVAALF, VYYTSAFVF, DIYRIFAEL, DVPSGKLFM, DIDLLLQRG, YVKVYLESF, TVQGQNLKY, EPMSIYVYAL, HVRVSQPSL, QARLTVSGL, RRRHRQDAL, QPKRRRHRQ, LCPKSIPGL, VLCPKNMII, YRIQGKLEY, SEHPTFTSQY, CEDVPSGKLF, NEIHNPAVF, RETVELRQY, QEPMSIYVY, LNIPSINVHHYPSAAERKHR, ATVQGQNLKYQEFFWDANDI, QEFFWDANDIYRIFAELEGV, PQYSEHPTFTSQYRIQGKLE, SQYRIQGKLEYRHTWDRHDE, VFTWPPWQAGILARNLVPMV, ILARNLVPMVATVQGQNLKY, DQYVKVYLESFCEDVPSGKL, YPSAAERKHRHLPVADAVIH, QYDPVAALFFFDIDLLLQRG, IIKPGKISHIMLDVAFTSHE, AHELVCSMENTRATKMQVIG, TRATKMQVIGDQYVKVYLES, MNGQQIFLEVQAIRETVELR, QAIRETVELRQYDPVAALFF, LTVSGLAWTRQQNQWKEPDV, WQPAAQPKRRRHRQDALPGP, YRHTWDRHDEGAAQGDDDVW, TSAGRKRKSASSATACTSGV, HRQDALPGPCIASTPKKHRG, YYTSAFVFPTKDVALRHVVC ,

VTTERKTPRVTGGGAMAGAS, QPFMRPHERNGFTVLCPKNM, SICPSQEPMSIYVYALPLKM, IYVYALPLKMLNIPSINVHH and YYTSAFVFPTKDVALRHVVC.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In further aspect the present invention consists in a T-cell epitope derived from IE-1 of HCMV, the T-cell epitope having a sequence selected from the group consisting of SLLSEFCRV, VLAELVKQI, ILGADPLRV, TMYGGISLL, LLSEFCRVL, VLEETSVML, CLQNALDIL, ILDEERDKV, IKEHMLKKY, DEEEAIVAY, CVETMCNEY, KLGGALQAK, QYILGADPL, KYTQTEEKF, KARAKKDEL, VMKRRIEEI, RHRIKEHML, ELRRKMMYM, QIKVRVDMV, CSPDEIMAYAQKIFKILDEE, EEAIVAYTLATAGVSSSDSL, SEPVSEIEEVAPEEEEDGAE and VLCCYVLEETSVMLAKRPLI.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In further aspect the present invention consists in a T-cell epitope derived from pp150 of HCMV, the T-cell epitope having a sequence selected from the group consisting of GQTEPIAFV, KMSVRETLV, FLGARSPSL, ALVNAVNKL, ALVNFLRHL, NILQKIEKI, LIEDFDIYV, PLIPTTAVI, RIEENLEGV, NVRRSWEEL, WPRERAWAL, KARDHLAVL, SPWAPTAPL, RPSTPRAAV, VKESPGRCY, LDEGIMVVY, ATSTGDVVY, NTDFRVLEL, AYIYTTYLL, SYENKTMQL, AYEYVDYLF, CYSRPVVIF, KMTATFLSK, IMREFNSYK, WPRERAWALKNPHLAYNPFR, QQLKAWEERQQNLQQRQQQP, SRDAADEVWALRDQTAESPV, VKPLDLNLDRHANTALVNAV, STSQKPVLGKRVATPHASAR and HANTALVNAVNKLVYTGRLI.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In further aspect the present invention consists in a T-cell epitope derived from gB of HCMV, the t-cell epitope having a sequence selected from the group consisting of RIWCLVVCV, QMLLALARL, GLDDLMSGL, IILVAIAVV, DLDEGIMVV, NLFPYLVSA, AVGGAVASV, YINRALAQI, RSYAYIYTTYLLGSNTEYVA, TYEKYGNVSVFETSGGLVVF, FETSGGLVVFWQGIKQKSLV, YHRDSYENKTMQLIPDDYSN, MQLIPDDYSNTHSTRYVTVK and LDEGIMVVYKRNIVAHTFKV.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In further aspect the present invention consists in a T-cell epitope derived from IE-2 of HCMV, the T-cell epitope having a sequence selected from the group consisting of FLMEHTMPV, LMQKFPKQV, NLALSTPFL, IIYTRNHEV, LLGALNLCL, KPEPDFTIQY, IIYTRNHEVK, IMKDKNTPF, PRKKKSKRI, TAAKAYAVGQFEQPTETPPE, FEQPTETPPEDLDTLSLAIE, MLPLIKQEDIKPEPDFTIQY, THQLCPRSSDYRNMIIHAAT, YRNMIIHAATPVDLLGALNL, TGPRKKKSKRISELDNEKVR, PVDLLGALNLCLPLMQKFPK, IQIIYTRNHEVKSEVDAVRC and VKSEVDAVRCRLGTMCNLAL.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In further aspect the present invention consists in a T-cell epitope derived from gH of HCMV, the T-cell epitope having a sequence selected from the group consisting of YLMDELRYV, YLTVFTVYL, TLTEDFFVV, LLMMSVYAL, YLLYRMLKT, ILFDGHDLL, LIFGHLPRV, SLVRLVYIL, LLYPTAVDL, ALDPYNEVV, LMLLKNGTV, SAIIGIYLL, ITSLVRLVY, HHEYLSDLY, AIIGIYLLY, QTEKHELLV, ATDSRLLMM, FLDAALDFNY, DTQGVINIMY, LRENTTQCTY, SAIIGIYLLY, SLRNSTVVR, ALALFAAAR, QLNRHSYLK, RLFPDATVP, AIIGIYLLY, RLNTYALVSK, LVRLVYILSK, YLMDELRYVK, ELYLMGSLVH, ALTVSEHVSY, NYLDLSALL, SYVVTNQYL, SYLKDSDFL, TYALVSKDL, SYRSFSQQL, TYGRPIRFL, YYVFHMPRCL, MYMHDSDDVL, ETFPDLFCL, DLTETLERY, SPRTHYLML, FPDLFCLPL, SPRTHYLMLL, MPRCLFAGPL, TPMLLIFGHL, APYQRDNFIL, GRCQMLDRR, RRDHSLERL, SEALDPHAF, RENTTQCTY, DDVLFALDPY, RQTEKHELLVLVKKAQLNRH and ALTVSEHVSYVVTNQYLIKG.

In a preferred embodiment of the present invention the T-cell epitope is a CTL epitope.

In further aspect the present invention consists in a CTL epitope derived from pp71 of HCMV, the CTL epitope having a sequence selected from the group consisting of QLLIPKSFTL, TLVIPSWHV, LLIPKSFTL, DLVPLTVSV, CSDPNTYIHK, EYIVQIQNAF and AEVVARHNPY.

In further aspect the present invention consists in a CTL epitope derived from pp28 of HCMV, the CTL epitope having a sequence selected from the group consisting of GLLGASMDL, PLREYLADL, FLLSHDAAL, LLLIVTPVV, LLVEPCARV, LLIDPTSGL, LVEPCARVY, GIKHEGLVK, ELLAGGRVF, RLLDLAPNY, ELLGRLNVY, CRYKYLRKK and ARVYEIKCR.

In further aspect the present invention consists in a CTL epitope derived from pp50 of HCMV, the CTL epitope having a sequence selected from the group consisting of QLRSVIRAL, LLNCAVTKL, VTEHDTLLY, RGDPFDKNY, GLDRNSGNY, TLLNCAVTK, TVRSHCVSK, TRVKRNVKK, YEQHKITSY and SEDSVTFEF.

In further aspect the present invention consists in a CTL epitope derived from US2 of HCMV, the CTL epitope having a sequence selected from the group consisting of TLLVLFIVYV, LLVLFIVYV and SMMWMRFFV.

In further aspect the present invention consists in a CTL epitope derived from US3 of HCMV, the CTL epitope having a sequence selected from the group consisting of LLFRTLLVYL, YLFSLVVLV and TLLVYLFSL.

In further aspect the present invention consists in a CTL epitope derived from UL18 of HCMV, the CTL epitope having the sequence TMWCLTLFV.

HCMV infection remains a significant clinical problem in the context of solid organ transplant and HIV-infection. It is now firmly established that reactivation of CMV infection may cause lethal organ dysfunction and is frequently seen in immunosuppressed individuals. Previous studies have shown that CD8+ cytotoxic T-cells (CTL) have a primary role in suppressing HCMV reactivation. Direct monitoring of CMV-specific CD8+ CTL using ELISPOT technology and/or HLA-peptide tetramer may prove to be of value in the management of patients after organ transplant and in HIV-infected individuals.

The present invention further provides a diagnostic kit based on a panel of HCMV T cell epitopes restricted through common HLA class I alleles (e.g. A1, A2, A3, A11, A24, B8, B27 and B44) to monitor T cell responses in transplant patients. These epitopes can be derived from pp65, IE-1, IE-2, pp150, gB and other antigens of HCMV and are preferrably the epitopes of the first aspect of the present invention.

Typically these epitopes will be provided as synthetic peptides to be used in an ELISPOT assay. Alternatively, these peptides can used to generate MHC-peptide tetramers. Both ELISPOT technology and tetramers can be used to longitudinally monitor T cell responses following transplant. Regular monitoring of T cells specific for appropriate HLA class I-restricted epitopes can be used to predict the minimal level of T cell responses required to block any virus-induced pathology.

Further information regarding ELISPOT assay and tetramer can be found in Schmittel A, Keilholz U, Thiel E, Scheibenbogen C. "Quantification of tumor-specific T lymphocytes with the ELISPOT assay." J Immunother 2000 May-Jun;23(3):289-95; and Singhal S, Shaw JC, Ainsworth J, Hathaway M, Gillespie GM, Paris H, Ward K, Pillay D, Moss PA, Mutimer DJ. "Direct visualization and quantitation of cytomegalovirus-specific CD8+ cytotoxic T-lymphocytes in liver transplant patients." Transplantation 2000 Jun 15;69(11):2251-9.

As used herein the term "subject" encompasses both human and non-human animals.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

All publications mentioned in the specification are herein incorporated by reference.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

In order that the nature of the present invention may be more clearly understood preferred forms will now be described with reference to the following non-limiting Examples.

Materials and Methods

Establishment and Maintenance of Cell Lines

Epstein-Barr virus-transformed lymphoblastoid cell lines (LCLs) were established from HCMV seropositive donors by exogenous virus transformation of peripheral B cells using the B95.8 and wil virus isolates. In addition, the peptide transporter (TAP)-negative B x T hybrid cell line 174 x CEM.T2 (referred to as T2) (Salter and Creswell, 1986) were used for peptide stabilisation assays. All cell lines were routinely maintained in RPMI 1640

supplemented with 2 mM L-glutamine, 100 IU/ml penicillin and 100 ug/ml streptomycin plus 10% foetal calf serum (FCS) (growth medium).

To generate phytohaemagglutinin (PHA) blasts, peripheral blood mononuclear (PBMC) cells were stimulated with PHA (Commonwealth Serum Laboratories, Melbourne) and after 3 days of culture, growth medium containing MLA 144 supernatant and recombinant IL2 (rIL-2) was added (Burrows *et. al.*, 1992). PHA blasts were propagated by twice-weekly replacement of IL-2 and MLA supernatant (no further PHA added) for up to 6 weeks.

Epitope Prediction and Peptide Synthesis

Two computer-based algorithm programs were used to predict putative HLA class I-restricted CTL epitopes from within the amino acid sequences of HCMV antigens pp65, pp71, pp150, pp28, pp50, IE-1, IE-2, gB, gH, US2, US3 and UL18. SYFPEITHI (http://www.uni-tuebingen.de/uni/kxi/) (Rammensee et. al., 1999) and the BioInformatics and Molecular Analysis Section (BIMAS) HLA Peptide Binding Predictions programs (http://bimas.dcrt.nih.gov/molbio/hla_bind) were used to predict 9-mer peptides capable of binding to Class I MHC molecules. Each peptide was assigned a score on the basis of the strength of the interaction between the MHC molecule and the peptide. Peptides that ranked higher than 23 in the SYFPEITHI program predictions and peptides that scored greater 100 from the BIMAS program predictions were synthesized using the Merrifield solid phase method (Valerio et. al., 1991) and purchased from Chiron Mimotopes (Melbourne, Australia). All peptides were dissolved in 10% dimethyl sulfoxide (DMSO) and diluted in serum-free RPMI 1640 medium for use in assays which tested for their ability to bind MHC molecules and induce both the production of interferon-γ (IFN-γ; ELISPOT) and CTL activity in donor PBMC and T cell clones.

MHC Stabilization Assay

The ability of synthetic peptides to stabilize MHC molecules on the surface of the T2 cell line was measured by indirect immunofluorescence as described perviously (Burrows et. al., 1996). Briefly, T2 cells (2×10^5) were incubated in serum-free AIM-V medium (Gibco Life Technologies, USA) in the presence of 5 μM of peptide for 1 hr at 37°C and 5% CO_2 in a humidified atmosphere. These were then incubated for a further 14-16 hrs at 26°C, after

which the cells were returned to 37°C for 2 hrs prior to immunofluorescent staining. Cells were washed free of unbound peptide with growth medium prior to the addition of primary antibody. Anti-HLA allele-specific monoclonal antibody was added to the T2 cells and incubated at 4°C for 30 min. After washing with growth medium, these cells were incubated with PE or FITC-labelled anti-mouse Ig-specific antibody (Silenus, AMRAD, Australia) at 4°C for 30 min. Finally cell were washed and resuspended in 500 µl of cold PBS supplemented with 1% FCS. A sample of T2 cells was incubated with AIM-V medium without peptide at 26°C for 14-16hrs and served as a negative control. The second negative control comprised a sample of T2 cells which had cultured in growth medium at 37°C without peptide.

ELISPOT assay

ELISPOT assay was used to assess whether PBMC from a large panel of seropositive donors, stimulated with HCMV peptides, could induce IFN-γ expression in T cells. Briefly, a 96 well nitrocellulose plate (Multiscreen, Millipore) were coated overnight at 4°C with mouse monoclonal antibody anti-IFN- IgG1 (10µg/ml; Mabtech Nacka, Sweden). The plate was then washed six times in Phosphate Buffered Saline (PBS) and blocked for 1 hour at 37°C with PBS supplemented with 5% FCS. The blocking solution was removed and PBMC from healthy HCMV seropositive donors were added at a concentration of 2.5×10^5 cells per well in RPMI + 10% FCS. These cells were incubated for 18 h at 37°C and 5% CO_2 in the presence of synthetic peptides from HCMV antigens (10µM). After incubation, the plate was washed three times with PBS supplemented with 0.05% Tween, followed by three washes with PBS alone. Biotinylated detection antibody, anti-IFN-γ (Mabtech, Nacka Sweden) was added to each well at a final concentration of 1µg/ml in PBS. The plates were incubated at room temperature for four hours and then washed, as described above. Streptavidin-Alkaline phosphatase (Sigma) was added to each well at a final concentration of 1µg/ml in PBS and incubated at room temperature in the dark for 2 hours. After a final wash with PBS, the substrate, 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium was added to each well and the plates incubated for 30 min at RT. Cells that produced IFN-γ in response to the presence of peptide were detected as purple spots on the nitrocellulose membrane of each well. The spots were counted automatically using a closed circuit camera (CCD) and ImagePro image analysis software (ImagePro, Huston, Texas). T cell

precursor frequencies (pCTL) for each peptide were based on the total number of PBMCs in the well and the number of peptide specific spots per well, over an average of 3 wells. The number of peptide-specific spots was also calculated by subtracting the negative control values, which consisted of PBMCs without peptide (an average of six wells), from the test wells.

Generation of Polyclonal and Clonal HCMV-specific CTLs

To generate polyclonal CTLs, $2x10^6$ PBMC from HCMV seropositive, healthy donors were co-cultivated for seven days with $1x10^6$ autologous PBMC sensitized with synthetic peptides (20μg/ml). On day 7, these lymphocytes were restimulated with peptide-sensitized autologous LCLs. After 10 days of culture in growth medium, the cells were used as polyclonal effectors in a standard ^{51}Cr-release assay against peptide-sensitized autologous PHA blasts.

To generate HCMV-specific CTL clones, PBMC (10^6/ml) were cultivated with peptide sensitized autologous lymphocytes (responder to stimulator ratio of 2:1) in 2ml culture wells for 3 days in growth medium. CTL clones, generated by seeding in 0.35% agarose, were established and maintained in growth medium containing highly purified recombinant human IL-2 from *E. coli* (Moss *et. al.*, 1988) and were restimulated once weekly with peptide-sensitized autologous LCLs. These CTL clones were screened on a panel of target cells either sensitized with synthetic peptides or infected with recombinant vaccinia virus encoding individual HCMV antigens (see below).

Vaccinia virus recombinants

Recombinant vaccinia constructs encoding HCMV antigens and a control vaccinia virus construct made by insertion of the pSC11 vector alone and negative for thymidine kinase (Vacc.TK$^-$) have been previously described (Riddell *et. al.*, 1988). Target cells were infected with recombinant vaccinia virus at a multiplicity of infection (MOI) of 10:1 for 1 h at 37°C, as described earlier (Khanna *et. al.*, 1991; Khanna *et. al.*, 1992). After overnight infection, cells were washed with growth medium and processed for CTL assays or for immunoblotting to assess the expression of recombinant HCMV antigens (Khanna *et. al.*, 1993).

Cytotoxicity Assay

Target cells were either infected with recombinant vaccinia viruses or pre-sensitized with synthetic peptide epitopes and then incubated with ^{51}Cr for 90 min. Following incubation, these cells were washed in growth medium and used as targets in a standard 5 h ^{51}Cr-release assay Moss *et. al.*, 1988).

Results and Discussion

HLA A2 Epitope Prediction and HLA Binding Peptides from HCMV Antigens

In the first set of experiments putative HLA A2-restricted CTL epitopes were identified using two computer-based algorithms, which predict 8, 9 or 10-mer amino acid sequences likely to bind successfully to MHC class I molecules, based on the half-time dissociation of the interaction. In addition, each peptide was assigned a score based on the strength of the interaction between the MHC molecule and the peptide. Peptides that ranked higher than 24 in the SYFPEITHI program and/or peptides that scored greater than 100 from the BIMAS program (70 in total; Table 1) were used to screen healthy, seropositive donors. All peptides predicted to bind HLA A2 were analysed for their capacity to bind to HLA class I MHC molecules and stabilize their expression on the surface of T2 cells. Data for the HLA A2 stabilization is shown in Figure 1. HCMV peptides showing significant increase in the relative fluorescence intensity, above that of the negative controls, for an individual HLA class I allele were considered as positive. A total of 70 HLA A2 binding predictive peptides from p65, IE-1, gH, pp71, gB, IE-2, pp150, pp28, pp50, US2, US3 and UL18 were tested in this assay and of these 58 peptides clearly showed strong HLA A2 binding (see Fig. 1). All peptides showing fluorscence intensity greater than mean + 3SD of the fluorescence intensity on T2 cells in the absence of peptide at 26°C were considered as strong binders.

ELISPOT assay on HLA A2 predictive peptides

To determine whether memory CTL responses against the predictive HCMV potential epitopes could be detected in healthy HLA A2-positive virus carriers, we used the ELISPOT assay, which allows rapid identification of CTL epitopes without prolonged *in vitro* culture. PBMC isolated from a panel of healthy virus carriers were stimulated with the peptides that gave a positive result in HLA binding assays, and the cells that produced IFN-γ were

detected. A total of 30 CTL epitopes were identified from the panel of 57 HLA A2-binding peptides. Comprehensive data on the ELISPOT assays are presented in Figure 2. Of these, 27 CTL epitopes have not previously been identified. Overall the T cell responses in ELISPOT assay indicated an interesting hierarchy between the different antigens of HCMV. As reported previously (Gyulai *et. al.*, 2000), pp65 was clearly the most dominant antigen recognised by all the healthy virus carriers (Fig. 2; panel A). The majority of the donors recognised two or more epitopes within this antigen. A range of precursor CTL frequencies for the pp65 epitopes were evident among the different donors (Fig. 2; panel A). These ranged from 29 spot forming cells (SFC)/10^6 PBMC to 1994 SFC/10^6 PBMC. The NLVPMVATV peptide from pp65 was the only epitope recognised by every donor tested in this study. Other commonly recognised epitopes from pp65 were RIFAELEGV (6/8 donors), LMNGQQIFL (5/8 donors) and MLNIPSINV (5/8 donors) (Fig. 2; panel A). For these four pp65 epitopes the average precursor frequency was highest for NLVPMVATV followed by LMNGQQIFL, MLNIPSINV and RIFAELEGV respectively. Occasional subdominant responses to other epitopes (RLLQTGIHV and ILARNLVPM) within pp65 were also detected by two different donors.

IE-1 was considered to be the second most dominant antigen, after pp65, followed by pp150, gB, gH, IE2, US2, pp28,US3 and pp50. 6/8 donors showed CTL reactivity to at least one CTL epitope within IE-1 (Fig. 2; panel B). Interestingly one of most dominant CTL responses to any epitope was identified within IE-1 (VLEETSVML). This epitope showed a precursor frequency of 3752 SFC/10^6 PBMC in the donor SB. It is important to mention here that this epitope overlaps with a previously published epitope within IE-1 (YILEETSVML) and both these epitopes were efficiently recognised by three different donors. Other novel epitopes identified from IE-1 (VLAELVKQI, SLLSEFCRV and CLQNALDIL) comparably showed much lower frequency. CTL frequencies to epitopes within gB, gH, pp28, pp50, US2, US3, IE-2 and pp150 were generally very low, although CTL epitopes within gB and gH were more frequently recognised by healthy virus carriers when compared to pp150, pp28, US2, US3 and pp50. Another fascinating observation of this analysis was the identification of IE-2, US2 and US3 antigens as a target for CTL response. To our knowledge this is the first report which has mapped novel epitopes within these antigens. Although only four HLA A2-positive healthy virus carriers were identified as potential responders to the epitopes

within these antigens, further screening for additional epitopes restricted through other HLA class I alleles have already identified a number of other epitopes within these antigens (See below).

Comparison of overall T cell reactivity to all the antigens amongst the panel of healthy virus carriers indicated that, generally, CTL responses to HCMV antigens are not constrained to a single antigen. The majority of the donors tested in our study showed a broad range of CTL responses to multiple antigens. Donors SB and CP showed the broadest range of CTL reactivity and, thus, recognised seven of the ten antigens tested in our study. Moreover both of these donors also recognised a greater number of epitopes (11/30) than any other donor tested in our study (Fig. 2). On average, CTL responses from our panel of healthy virus carriers were directed towards 6-7 different epitopes. An overall summary of the HLA A2 binding and ELISPOT assays is shown in Table 2. These results strongly suggest that broadly directed CTL responses to multiple epitopes may be essential in controlling the HCMV replication. Indeed, recent studies with other human viral infections have also indicated that individuals with broad T cell reactivity were able to clear the virus infection more efficiently than those who displayed a more narrowly focussed CTL response to a single antigen, or a limited number of eptiopes. These observations further highlight the importance of designing a HCMV vaccine that combines in one single regimen all those antigens that might provide protection.

Analysis of HCMV-Specific CTL Responses Using Cytotxicity Assays

To further confirm our CTL epitopes identified by ELISPOT assays, we generated polyclonal and clonal CTL lines specific for these epitopes. PBMC from the donors SB, WS, CP and BS were stimulated with synthetic peptide epitopes, and the CTL clones or polyclonal lines that were established were tested in standard 51Cr-release assays. Representative data from the polyclonal CTL lines established from the donor SB are shown in Figure 3. A total of seven different HLA A2-restricted epitopes from IE-2, pp150, pp65, IE-1 and gB antigens were assessed in these assays. Of these, 6 epitopes recalled strong CTL responses, while a low level of CTL activity was observed in the polyclonal CTL line established against the GQTEPIAFV (pp150) epitope. It is important to emphasise here that, generally, the overall strength of CTL activity correlated with the number IFN-γ-producing cells seen in the ELISPOT assays. For example, VLEETSVML epitope from IE-1 antigen,

which was the most dominant epitope for the donor SB in the ELISPOT assays, also showed strong CTL activity in the cytotoxicity assays (Fig. 2 and 3).

In addition, a series of CTL clones were established to further characterise these epitopes. Representative data from three different epitopes is presented in Figure 4 (panel A-C). Of particular interest was the CTL clone specific for the VLEETSVML epitope from IE-1 antigen. Previous studies by Retiere and colleagues (2000) have identified another HLA A2-restricted epitope (YILEETSVM) within IE-1 antigen which is very similar to the VLEETSVML epitope mapped in our study. To determine whether the CTL response to VLEETSVML epitope is identical to that seen for YILEETSVM, we titrated both these peptides and compared the cytotoxic activity in a standard ^{51}Cr-release assay. The data presented in Figure 4; panel A clearly shows that CTL clone specific for VLEETSVML epitope only recognises the VLEETSVML peptide, while YILEETSVM is very poorly recognised. These observations have also been confirmed by polyclonal CTL lines established from other HLA A2-positive donors (data not shown). Thus it is clear from these analyses that both VLEETSVML and YILEETSVM represent distinct CTL epitopes. Other CTL clones tested in this study were specific for NLVPMVATV epitope from pp65 (Fig. 4; panel B) and IIYTRNHEV epitope from IE-2 antigen (Fig. 4; panel C). Comparison of overall CTL reactivity of clones specific for IIYTRNHEV revealed that generally this epitope was ~~very~~ poorly recognised at limiting concentrations (Fig. 4; panel C). The peptide concentration required for half maximal lysis for the IIYTRNHEV epitope was almost 100-500 fold more than the NLVPMVATV and VLEETSVML epitopes, respectively. An identical pattern of peptide titration was also seen with another five different CTL clones specific for the epitope IIYTRNHEV (data not shown).

Epitope Prediction and Functional Binding Analysis of Peptide Epitopes from HCMV Antigens Restricted Through Other Common HLA Class I Alleles

Having successfully developed a novel strategy to rapidly map a large cohort of HLA A2-restricted CTL epitopes from HCMV antigens, we extended our analysis to identify epitopes restricted through other HLA class I alleles which are commonly prevalent in different ethnic groups. These HLA class I alleles included HLA A1, A3, A24, A26, B7, B8, B27 and B44. As in the case of HLA A2, a number of potential epitopes were identified using two

computer-based algorithms which assigned a score based on the strength of the interaction between the MHC molecule and the peptide. Peptides that ranked higher than 24 in the SYFPEITHI program and/or peptides that scored greater than 100 from the BIMAS program were used to screen healthy, seropositive donors. A total of 118 epitopes were predicted to bind different HLA class I molecules (Table 3). Synthetic peptides representing each of these epitopes were analysed for their capacity to bind to HLA class I molecules and stabilise their expression on the surface of T2 cells. For these assays, T2 cells transfected with cDNA encoding individual HLA class I allele (A3, B7, B8 and B27) were used. Representative data for the stabilisation of HLA B7 are shown in Figure 5. HCMV peptides showing a significant increase in relative fluorescence intensity, above that of the negative controls, for an individual HLA class I allele were considered as positive. A total of 52 HLA A3-, B7-, B8-, and B27-binding, predictive peptides from pp28, pp50, pp71, p65, pp150, IE1, IE2, gB and gH were tested in these assays, and of these, 41 peptides clearly showed strong binding (see Table 4). Stabilisation for HLA A1, A24, A26 and B44 binding predictive peptides was not tested in these assays.

ELISPOT assay on other HLA class I predictive peptides

In the next set experiments we tested all these predictive epitopes in ELISPOT assays to determine whether memory T cell responses could be detected in healthy HLA-matched virus carriers. PBMC from a panel of healthy virus carriers (HLA A1, A3, A24, B7, B8 and/or B27) were stimulated with individual peptides and the cells that produced IFN-γ were detected. A total 23 CTL epitopes restricted through either HLA A1, A3, A24, B7, B8 or B27 were identified from a panel of 111 peptides tested in these assays. A summary of these ELISPOT results, along with the HLA class I binding data, is presented in Table 4 and representative data showing the ELISPOT responses to HLA B8, B7, A24, B27 and A1-restricted epitopes are shown in Figure 6. As in the case of HLA A2-restricted CTL responses, both pp65 and IE-1 were clearly the most immunodominant antigens. One of the surprising aspects of the CTL responses to IE-1 epitopes was that, in some individuals, this response constituted 5-10% of their total CD8+ T cell population (data not shown). Other antigens such as pp150, gH, pp28 and pp50 were also identified as potential targets for class I-restricted CTL response (Fig. 6). Although pp28 and pp50 have been identified as potential targets of CTL

response, to our knowledge, this is the first report of multiple CTL epitopes restricted through HLA A1, A2 and B27 within these antigens have been identified.

ELISPOT assays using overlapping peptides from HCMV antigens

Although the computer-based algorithms were successfully employed to map a large panel of novel epitopes, we also tested a panel of overlapping peptides from six different antigens of HCMV (gB, gH, IE-1, IE-2, pp150 and pp65) to determine whether memory T cell responses could be detected in healthy virus carriers. A total of 500 peptides were tested in each assay. This analysis was primarily aimed at mapping CTL epitopes for those alleles where prediction algorithms were not available. PBMC from a panel of healthy virus carriers were stimulated with individual peptides and the cells that produced IFN-γ were detected. A summary of these ELISPOT assays is presented in Table 5. Strong ELISPOT responses to 54 peptides from different HCMV antigens were detected. Consistent with our earlier analysis, peptides from pp65 were the most frequently recognised epitopes in ELISPOT assays. Peptides from other antigens were less frequently recognised. Interestingly, peptides from IE-2 antigen were more frequently recognised when compared to IE-1, gB, gH and pp150. Minimal sequences within some of these 20 amino acid peptides were mapped by ELISPOT assays using overlapping peptides. These minimal sequences are also shown in Table 5.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this 26th day of June 2001

The Council of the Queensland
Institute of Medical Research
Patent Attorneys for the Applicant:

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Table 1: Predicted HLA A2-restricted CTL epitopes within HCMV antigens

HCMV ANTIGEN	Amino Acid Sequence	Binding Score	
		NIH-BIMAS[i]	SYFPEITHI[ii]
pp65	QMWQARLTV	416.926	
	ALFFFDIDL	300.355	
	RLLQTGIHV*	257.342	
	NLVPMVATV*	159.970	30
	LMNGQQIFL	144.256	
	MLNIPSINV*	118.238	
	RIFAELEGV		24
	ILARNLVPM*		24
	VIGDQYVKV		24
IE-1	SLLSEFCRV	488.951	24
	VLAELVKQI	167.248	27
	ILGADPLRV	188.238	26
	TMYGGISLL	113.535	27
	LLSEFCRVL		25
	VLEETSVML		24
	CLQNALDIL		24
	ILDEERDKV	95.016	26
pp150	GQTEPIAFV	365.74	
	KMSVRETLV	175.81	
	FLGARSPSL		24
	ALVNAVNKL		29
	ALVNFLRHL		29
	NILQKIEKI		27
	LIEDFDIYV		24
	PLIPTTAVI		24
	RIEENLEGV		24

Table 1 (cont)

HCMV ANTIGEN	Amino Acid Sequence	Binding Score	
		NIH-BIMAS[iii]	SYFPEITHI[iv]
gB	RIWCLVVCV	182.565	26
	QMLLALARL	131.296	26
	GLDDLMSGL		24
	IILVAIAVV		28
	DLDEGIMVV		27
	NLFPYLVSA		26
	AVGGAVASV		26
	YINRALAQI		25
IE-2	FLMEHTMPV	4853.478	26
	LMQKFPKQV	324.499	
	NLALSTPFL	117.493	25
	IIYTRNHEV		24
	LLGALNLCL		24
gH	YLMDELRYV	12279.29	28
	YLTVFTVYL	2053.781	26
	TLTEDFFVV	1288.424	
	LLMMSVYAL	309.050	29
	YLLYRMLKT	291.716	
	ILFDGHDLL	269.948	26
	LIFGHLPRV	138.354	28
	SLVRLVYIL		28
	LLYPTAVDL		27
	ALDPYNEVV		25
	LMLLKNGTV		25
	SAIIGIYLL		25
pp71	QLLIPKSFTL	151.648	
	TLVIPSWHV	521.640	
	LLIPKSFTL	210.633	29
	DLVPLTVSV		28

Table 1 (cont)

HCMV ANTIGEN	Amino Acid Sequence	Binding Score	
		NIH-BIMAS[v]	SYFPEITHI[vi]
pp28	LLIDPTSGL		29
	LLVEPCARV		27
	LLLIVTPVV		27
	FLLSHDAAL		26
	PLREYLADL		26
	GLLGASMDL		25
pp50	LLNCAVTKL		29
	QLRSVIRAL		25
US2/ US3/ UL18	TLLVLFIVYV		
	LLVLFIVYV		
	SMMWMRFFV		
	LLFRTLLVYL		
	YLFSLVVLV		
	TLLVYLFSL		
	TMWCLTLFV		

[v]The algorithm used to score runs as follows: The initial (running) score is set to 1.0. For each residue position, the program examines which amino acid is appearing at that position. The running score is then multiplied by the coefficient for that amino acid type, at that position, for the chosen HLA molecule. These coefficients have been pre-calculated and are stored for use by the scoring algorithm in a separate directory as a collection of HLA coefficient files. Using 9-mers, nine multiplications are performed. The resulting running score is multiplied by a final constant to yield an estimate of the half time of disassociation. The final multiplication yields the score reported in the output table. . Further explanations on the algorithm can be found in: Parker et. al. J. Immunol. 1994. 152:163-175.

[vi]The scoring system evaluates every amino acid within a given peptide. Individual amino acids may be given the arbitrary value 1 for amino acids that are only slightly preferred in the respective position, optimal anchor residues are given the value 15; any value between these two is possible. Negative values are also possible for amino acids which are disadvantageous for the peptide's binding capacity at a certain sequence position. The allocation of values is based on the frequency of the respective amino acid in natural ligands, T-cell epitopes, or binding peptides. Further explanations on the algorithm can be found in: Rammensee et. al. Immunogenetics 1999. 50: 213-219.

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Table 2: Summary of HLA binding, ELISPOT and cytotoxicity assays on HLA A2 predicted CTL epitopes from HCMV antigens

ANTIGEN	Amino Acid Sequence	HLA A2 Binding[vii]	ELISPOT[viii]	CTL Activity[ix]
pp65	QMWQARLTV	+	-	
	ALFFFDIDL	++	-	
	RLLQTGIHV	++	+	NT
	NLVPMVATV	+++	++++	Positive
	LMNGQQIFL	++	++	NT
	MLNIPSINV	++	++	NT
	RIFAELEGV	++	+	NT
	ILARNLVPM	++	+	NT
	VIGDQYVKV	++	-	
IE-1	SLLSEFCRV	+++	++	
	VLAELVKQI	++	++	Positive
	ILGADPLRV	+++	-	
	TMYGGISLL	++	-	NT
	LLSEFCRVL	++	-	
	VLEETSVML	+++	+++++	Positive
	YILEETSVM	++	+++	Positive
	CLQNALDIL	++	+	
	ILDEERDKV	+++	-	
pp150	GQTEPIAFV	++	+	Weak Positive
	KMSVRETLV	++	+	
	FLGARSPSL	++	-	
	ALVNAVNKL	++	+	
	ALVNFLRHL	++	+	
	NILQKIEKI	++	-	NT
	LIEDFDIYV	++	+	
	PLIPTTAVI	-	-	
	RIEENLEGV	+	-	

Table 2 (cont)

ANTIGEN	Amino Acid Sequence	HLA A2 Binding[x]	ELISPOT[xi]	CTL Activity[xii]
gB	RIWCLVVCV	+	+	NT
	QMLLALARL	++	-	
	GLDDLMSGL	++	-	
	IILVAIAVV	++	-	
	DLDEGIMVV	+	-	
	NLFPYLVSA	++	-	
	AVGGAVASV	-	++	Positive
	YINRALAQI	++	-	
IE-2	FLMEHTMPV	++	+	
	LMQKFPKQV	+	-	
	NLALSTPFL	++	-	
	IIYTRNHEV	++	++	Positive
	LLGALNLCL	+++	-	
gH	YLMDELRYV	++	-	
	YLTVFTVYL	+++	-	
	TLTEDFFVV	+++	-	
	LLMMSVYAL	++	-	
	YLLYRMLKT	+	-	
	ILFDGHDLL	+++	-	
	LIFGHLPRV	+++	+	NT
	SLVRLVYIL	++	-	
	LLYPTAVDL	+++	-	
	ALDPYNEVV	+++	-	
	LMLLKNGTV	-	+	NT
	SAIIGIYLL	-	-	
pp71	QLLIPKSFTL	+	-	
	TLVIPSWHV	++	-	
	LLIPKSFTL	+	-	
	DLVPLTVSV	+	-	NT

Table 2 (cont)

ANTIGEN	Amino Acid Sequence	HLA A2 Binding [xiii]	ELISPOT[xiv]	CTL Activity[xv]
pp28	GLLGASMDL	+	-	
	PLREYLADL	+	-	
	FLLSHDAAL	+++	-	
	LLLIVTPVV	+++	-	
	LLVEPCARV	+++	-	
	LLIDPTSGL	++	+	NT
pp50	QLRSVIRAL	-	-	
	LLNCAVTKL	+++	+	NT
US2/ US3/ UL18	TLLVLFIVYV	-	+/-	NT
	LLVLFIVYV	-	+/-	NT
	SMMWMRFFV	+++	+	NT
	LLFRTLLVYL	+++	+	NT
	YLFSLVVLV	+	+	NT
	TLLVYLFSL	+++	-	
	TMWCLTLFV	+++	-	

[1] The levels of HLA A2 expression in the presence of peptide are expressed relative to the HLA A2 expression on T2 cells in the absence of peptide at 26°C: +++ = 200-300%, ++ = 100-199%, + = 51-99%, - = 0-50%, relative increase in HLA A2 expression.

[1] The number of IFNγ producing cells following peptide stimulation in an ELISPOT assay are expressed number of spot forming cells (SFC) per $1x10^6$ PBMC: +++++ 2000-4000 SFC, ++++ =1000-1999 SFC, +++ = 500-999 SFC, ++ = 100- 499 SFC, + = 20-99 SFC, - = 0-19 SFC.

[3]CTL Activity measured as % specific lysis in a standard 51Cr-release assay

Table 3: Predicted HLA Class I-restricted CTL epitopes from HCMV antigens

ANTIGEN	Amino Acid Sequence	HLA Restriction	Binding Score	
			NIH-BIMAS[xvi]	SYFPEITHI[xvii]
pp65	SQEPMSIYVY	HLA-A1		24
	ATVQGQNLKY	HLA-A1		23
	IRETVELRQY	HLA-A1		24
	IGDQYVKVY	HLA-A1		28
	TVQGQNLKY	HLA-A1		27
	YRIQGKLEY	HLA-A1		25
	QVIGDQYVK	HLA-A3		24
	LLLQRGPQY	HLA-A3		24
	RVTGGGAMA	HLA-A3		25
	GVMTRGRLK	HLA-A3		25
	VYALPLKML	HLA-A24	200.000	
	QYDPVAALF	HLA-A24	168.000	
	VYYTSAFVF	HLA-A24	100.000	
	DIYRIFAEL	HLA-A26		30
	DVPSGKLFM	HLA-A26		26
	DIDLLLQRG	HLA-A26		24
	YVKVYLESF	HLA-A26		26
	TVQGQNLKY	HLA-A26		24
	EPMSIYVYAL	HLA-B7		24
	HVRVSQPSL	HLA-B7	200.00	
	QARLTVSGL	HLA-B7	120.000	
	RRRHRQDAL	HLA-B27/B8	6000.00	26
	QPKRRRHRQ	HLA-B8		24
	LCPKSIPGL	HLA B8		
	VLCPKNMII	HLA-B8		24
	YRIQGKLEY	HLA-B27	1000.00	27
	SEHPTFTSQY	HLA-B44	720.00	
	CEDVPSGKLF	HLA-B44		
	NEIHNPAVF	HLA-B44	200.00	
	RETVELRQY	HLA-B44	180.00	
	QEPMSIYVY	HLA-B44	120.00	

Table 3 (cont)

ANTIGEN	Amino Acid Sequence	HLA Restriction	Binding Score	
			NIH-BIMAS[xviii]	SYFPEITHI[xix]
IE-1	IKEHMLKKY	HLA-A1		27
	DEEEAIVAY	HLA-A1		25
	CVETMCNEY	HLA-A1		25
	KLGGALQAK	HLA-A3	135.00	
	QYILGADPL	HLA-A24	300.00	
	KYTQTEEKF	HLA-A24	220.00	
	KARAKKDEL	HLA-B7/B8	120.00/160.00	
	VMKRRIEEI	HLA-B8		27
	RHRIKEHML	HLA-B8		26
	ELRRKMMYM	HLA-B8/A26		24
	QIKVRVDMV	HLA-B8		24
	ELKRKMMYM*	HLA-B8	Known Epitope	
	RRKMMYMCY*	HLA-B27	Known Epitope	
	RRIEEICMK*	HLA-B27	Known Epitope	
pp150	NVRRSWEEL	HLA-B7	200.00	
	WPRERAWAL	HLAB7/B8/B35	800.00	
	KARDHLAVL	HLA-B7	120.00	
	SPWAPTAPL	HLA-B7	120.00	27
	RPSTPRAAV	HLA-B7		25
	VKESPGRCY	HLA-A1		28
	LDEGIMVVY	HLA-A1		27
	ATSTGDVVY	HLA-A1		25
	NTDFRVLEL	HLA-A1		23
	AYIYTTYLL	HLA-A24	300.00	
	SYENKTMQL	HLA-A24	300.00	
	AYEYVDYLF	HLA-A24	210.00	

Table 3 (cont)

ANTIGEN	Amino Acid Sequence	HLA Restriction	Binding Score	
			NIH-BIMAS[xx]	SYFPEITHI[xxi]
pp150	CYSRPVVIF	HLA-A24	100.00	
	KMTATFLSK	HLA-A3	360.00	
	IMREFNSYK	HLA-A3	90.00	24
IE-2	KPEPDFTIQY	HLA-A1	562.50	33
	IIYTRNHEVK	HLA-A3		28
	IMKDKNTPF	HLA-B8		30
	PRKKKSKRI	HLA-B8		27
gH	ITSLVRLVY	HLA-A1		
	HHEYLSDLY	HLA-A1		27
	AIIGIYLLY	HLA-A1/A3/A26		
	QTEKHELLV	HLA-A1		24
	ATDSRLLMM	HLA-A1		24
	FLDAALDFNY	HLA-A1	125.00	
	DTQGVINIMY	HLA-A1		24
	LRENTTQCTY	HLA-A1		24
	SAIIGIYLLY	HLA-A1		24
	SLRNSTVVR	HLA-A3		27
	ALALFAAAR	HLA-A3		24
	QLNRHSYLK	HLA-A3		25
	RLFPDATVP	HLA-A3		25
	RLNTYALVSK	HLA-A3		24
	LVRLVYILSK	HLA-A3		29
	YLMDELRYVK	HLA-A3		26
	ELYLMGSLVH	HLA-A3		26
	ALTVSEHVSY	HLA-A3		25
	NYLDLSALL	HLA-A24	432.00	
	SYVVTNQYL	HLA-A24	360.00	
	SYLKDSDFL	HLA-A24	300.00	
	TYALVSKDL	HLA-A24	280.00	
	SYRSFSQQL	HLA-A24	240.00	

Table 3 (cont)

ANTIGEN	Amino Acid Sequence	HLA Restriction	Binding Score	
			NIH-BIMAS[xxii]	SYFPEITHI[xxiii]
gH	TYGRPIRFL	HLA-A24	200.00	
	YYVFHMPRCL	HLA-A24	300.00	
	MYMHDSDDVL	HLA-A24	300.00	
	ETFPDLFCL	HLA-A26		32
	DLTETLERY	HLA-A26		31
	SPRTHYLML	HLA-B7	800.00	24
	FPDLFCLPL	HLA-B7		24
	SPRTHYLMLL	HLA-B7	800.00	22
	MPRCLFAGPL	HLA-B7	800.00	23
	TPMLLIFGHL	HLA-B7	240.00	22
	APYQRDNFIL	HLA-B7	240.00	22
	GRCQMLDRR	HLA-B27		28
	RRDHSLERL	HLA-B27		28
	SEALDPHAF	HLA-B44	160.00	
	RENTTQCTY	HLA-B44	120.00	
	DDVLFALDPY	HLA-B44	202.500	
pp71	CSDPNTYIHK	HLA-A1	750.00	22
	EYIVQIQNAF	HLA-A24	252.00	
	AEVVARHNPY	HLA-B44	240.00	
pp28	LVEPCARVY	HLA-A1/A3		30/26
	GIKHEGLVK	HLA-A3		29
	ELLAGGRVF	HLA-A3		27
	RLLDLAPNY	HLA-A3		26
	ELLGRLNVY	HLA-A3		26
	CRYKYLRKK	HLA-B2705		27
	ARVYEIKCR	HLA-B2705		26

Table 3 (cont)

ANTIGEN	Amino Acid Sequence	HLA Restriction	Binding Score	
			NIH-BIMAS[xxiv]	SYFPEITHI[xxv]
pp50	VTEHDTLLY	HLA-A1		40
	RGDPFDKNY	HLA-A1		29
	GLDRNSGNY	HLA-A1		26
	TLLNCAVTK	HLA-A3		34
	TVRSHCVSK	HLA-A3		29
	TRVKRNVKK	HLA-B27		27
	YEQHKITSY	HLA-B44	270.00	
	SEDSVTFEF	HLA-B44	270.00	

[i]The algorithm used to score runs as follows: The initial (running) score is set to 1.0. For each residue position, the program examines which amino acid is appearing at that position. The running score is then multiplied by the coefficient for that amino acid type, at that position, for the chosen HLA molecule. These coefficients have been pre-calculated and are stored for use by the scoring algorithm in a separate directory as a collection of HLA coefficient files. Using 9-mers, nine multiplications are performed. The resulting running score is multiplied by a final constant to yield an estimate of the half time of disassociation. The final multiplication yields the score reported in the output table. . Further explanations on the algorithm can be found in: Parker et. al. J. Immunol. 1994. 152:163-175.

[ii]The scoring system evaluates every amino acid within a given peptide. Individual amino acids may be given the arbitrary value 1 for amino acids that are only slightly preferred in the respective position, optimal anchor residues are given the value 15; any value between these two is possible. Negative values are also possible for amino acids which are disadvantageous for the peptide's binding capacity at a certain sequence position. The allocation of values is based on the frequency of the respective amino acid in natural ligands, T-cell epitopes, or binding peptides. Further explanations on the algorithm can be found in: Rammensee et. al. Immunogenetics 1999. 50: 213-219.

* PUBLISHED EPITOPES

Table 4: Summary of HLA binding and ELISPOT assays CTL epitopes from HCMV antigens

ANTIGEN	Amino Acid Sequence	HLA Restriction	Functional Activity	
			HLA Binding [1]	ELISPOT[2]
pp65	SQEPMSIYVY	HLA-A1	NT	-
	ATVQGQNLKY	HLA-A1	NT	-
	IRETVELRQY	HLA-A1	NT	-
	IGDQYVKVY	HLA-A1	NT	-
	TVQGQNLKY	HLA-A1	NT	-
	YRIQGKLEY	HLA-A1	NT	-
	QVIGDQYVK	HLA-A3	-	-
	LLLQRGPQY	HLA-A3	++	-
	RVTGGGAMA	HLA-A3	-	-
	GVMTRGRLK	HLA-A3	++	-
	VYALPLKML	HLA-A24	NT	-
	QYDPVAALF	HLA-A24	NT	-
	VYYTSAFVF	HLA-A24	NT	-
	DIYRIFAEL	HLA-A26	NT	NT
	DVPSGKLFM	HLA-A26	NT	NT
	DIDLLLQRG	HLA-A26	NT	NT
	YVKVYLESF	HLA-A26	NT	NT
	TVQGQNLKY	HLA-A26	NT	NT
	EPMSIYVYAL	HLA-B7	++	NT
	HVRVSQPSL	HLA-B7	++	+++
	TPRVTGGGGAM*	HLA B7	+++	+++
	RPHERNGFTVL*	HLA B7	+++	++
	QARLTVSGL	HLA-B7	+++	+++
	RRRHRQDAL	HLA-B27/B8	++	-
	QPKRRRHRQ	HLA-B8	-	-
	LCPKSIPGL	HLA B8	+	++++
	VLCPKNMII	HLA-B8	+	++++

Table 4 (cont)

ANTIGEN	Amino Acid Sequence	HLA Restriction	Functional Activity	
			HLA Binding [1]	ELISPOT[2]
pp65	YRIQGKLEY	HLA-B27	++	-
	SEHPTFTSQY	HLA-B44	NT	NT
	CEDVPSGKLF	HLA-B44	NT	NT
	NEIHNPAVF	HLA-B44	NT	NT
	RETVELRQY	HLA-B44	NT	NT
	QEPMSIYVY	HLA-B44	NT	NT
IE-1	IKEHMLKKY	HLA-A1	NT	-
	DEEEAIVAY	HLA-A1	NT	-
	CVETMCNEY	HLA-A1	NT	-
	KLGGALQAK	HLA-A3	++	+++
	QYILGADPL	HLA-A24	NT	-
	KYTQTEEKF	HLA-A24	NT	-
	KARAKKDEL	HLA-B7/B8	++/+	+++++
	VMKRRIEEI	HLA-B8	+++	++++
	RHRIKEHML	HLA-B8	+	+++++
	ELRRKMMYM	HLA-B8/A26	+++	+++++
	QIKVRVDMV	HLA-B8	+++	-
	ELKRKMMYM*	HLA-B8	+++	+++++
	RRKMMYMCY*	HLA-B27	+++	-
	RRIEEICMK*	HLA-B27	-	-
pp150	NVRRSWEEL	HLA-B7	++	++++
	WPRERAWAL	HLAB7/B8/B35	++/++/NT	-/-/-
	KARDHLAVL	HLA-B7	+++	+++
	SPWAPTAPL	HLA-B7	+++	++
	RPSTPRAAV	HLA-B7	+++	++
	VKESPGRCY	HLA-A1	NT	-
	LDEGIMVVY	HLA-A1	NT	-
	ATSTGDVVY	HLA-A1	NT	-
	NTDFRVLEL	HLA-A1	NT	-
	AYIYTTYLL	HLA-A24	NT	-

Table 4 (cont)

ANTIGEN	Amino Acid Sequence	HLA Restriction	Functional Activity	
			HLA Binding [1]	ELISPOT[2]
pp150	SYENKTMQL	HLA-A24	NT	-
	AYEYVDYLF	HLA-A24	NT	-
	CYSRPVVIF	HLA-A24	NT	-
	KMTATFLSK	HLA-A3	++	-
	IMREFNSYK	HLA-A3	++	-
IE-2	KPEPDFTIQY	HLA-A1	NT	-
	IIYTRNHEVK	HLA-A3	++	-
	IMKDKNTPF	HLA-B8	+++	-
	PRKKKSKRI	HLA-B8	-	-
gH	ITSLVRLVY	HLA-A1	NT	-
	HHEYLSDLY	HLA-A1	NT	-
	AIIGIYLLY	HLA-A1	NT	-
	QTEKHELLV	HLA-A1	NT	-
	ATDSRLLMM	HLA-A1	NT	-
	FLDAALDFNY	HLA-A1	NT	-
	DTQGVINIMY	HLA-A1	NT	-
	LRENTTQCTY	HLA-A1	NT	-
	SAIIGIYLLY	HLA-A1	NT	-
	SLRNSTVVR	HLA-A3	++	+
	ALALFAAAR	HLA-A3	-	-
	QLNRHSYLK	HLA-A3	++	-
	RLFPDATVP	HLA-A3	-	+
	AIIGIYLLY	HLA-A3/A26	+	-
	RLNTYALVSK	HLA-A3	++	-
	LVRLVYILSK	HLA-A3	+++	-
	YLMDELRYVK	HLA-A3	-	-
	ELYLMGSLVH	HLA-A3	-	-
	ALTVSEHVSY	HLA-A3	NT	-
	NYLDLSALL	HLA-A24	NT	
	SYVVTNQYL	HLA-A24	NT	+
	SYLKDSDFL	HLA-A24	NT	-
	TYALVSKDL	HLA-A24	NT	-

Table 4 (cont)

ANTIGEN	Amino Acid Sequence	HLA Restriction	Functional Activity	
			HLA Binding [1]	ELISPOT[2]
gH	SYRSFSQQL	HLA-A24	NT	-
	TYGRPIRFL	HLA-A24	NT	-
	YYVFHMPRCL	HLA-A24	NT	-
	MYMHDSDDVL	HLA-A24	NT	-
	ETFPDLFCL	HLA-A26	NT	NT
	DLTETLERY	HLA-A26	NT	NT
	SPRTHYLML	HLA-B7	+++	-
	FPDLFCLPL	HLA-B7	+++	-
	SPRTHYLMLL	HLA-B7	+++	-
	MPRCLFAGPL	HLA-B7	+++	-
	TPMLLIFGHL	HLA-B7	+++	-
	APYQRDNFIL	HLA-B7	+++	-
	GRCQMLDRR	HLA-B27	-	-
	RRDHSLERL	HLA-B27	-	+
	SEALDPHAF	HLA-B44	NT	-
	RENTTQCTY	HLA-B44	NT	-
	DDVLFALDPY	HLA-B44	NT	-
pp71	CSDPNTYIHK	HLA-A1	NT	-
	EYIVQIQNAF	HLA-A24	NT	-
	AEVVARHNPY	HLA-B44	NT	NT
pp28	LVEPCARVY	HLA-A1/A3	NT	-
	GIKHEGLVK	HLA-A3	NT	-
	ELLAGGRVF	HLA-A3	NT	-
	RLLDLAPNY	HLA-A3	NT	-
	ELLGRLNVY	HLA-A3	NT	-
	CRYKYLRKK	HLA-B*2705	NT	-
	ARVYEIKCR	HLA-B*2705	NT	+

Table 4 (cont)

ANTIGEN	Amino Acid Sequence	HLA Restriction	Functional Activity	
			HLA Binding [1]	ELISPOT[2]
pp50	VTEHDTLLY	HLA-A1	NT	+++
	RGDPFDKNY	HLA-A1	NT	-
	GLDRNSGNY	HLA-A1	NT	-
	TLLNCAVTK	HLA-A3	NT	-
	TVRSHCVSK	HLA-A3	NT	-
	TRVKRNVKK	HLA-B27	NT	-
	TRLSEPPTL	HLA-B27	NT	-
	YEQHKITSY	HLA-B44	NT	++
	SEDSVTFEF	HLA-B44	NT	NT

[1] The levels of HLA A2 expression in the presence of peptide are expressed relative to the HLA class I expression on T2 cells in the absence of peptide at 26°C: + + + = 200-300%, + + = 100-199%, + = 51-99%, - = 0-50%, relative increase in HLA A2 expression.

[2] The number of IFNγ producing cells following peptide stimulation in an ELISPOT assay are expressed number of spot forming cells (SFC) per $1x10^6$ PBMC: + + + + + 2000-4000 SFC, + + + + =1000-1999 SFC, + + + = 500-999 SFC, ++ = 100- 499 SFC, + = 20-99 SFC, - = 0-19 SFC.

* PUBLISHED EPITOPES

TABLE 5: SUMMARY OF ELISPOT ANALYSIS BASED ON OVERLAPPING 20mer PEPTIDES FROM HCMV ANTIGENS

Antigen	Peptide Sequence	Peptide ID	Donor and HLA Type	Elispot Results (with 20mer)	Minimal sequence defined (CTLp with ELISPOT)
gB	RSYAYIYTTYLLGSNTEYVA	0B6	RK (A23, A24, B27, B41)	170	YAYIYTTYLLGS (75)
	TYEKYGNVSVFETSGGLVVF	0D8	SE(A2, A29, B44, B60) MB(A1, A24, B7, B58)	186 155	KYGNVSVFETSG (77; MB)
	FETSGGLVVFWQGIKQKSLV	0D9	MB(A1, A24, B7, B58)	23	
			SE(A2, A29, B44, B60)	167	
			SB(A2, B35, B57)	185	
	YHRDSYENKTMQLIPDDYSN	0B11			
	MQLIPDDYSNTHSTRYVTVK	0B12	MB(A1, A24, B7, B58)	238	DDYSNTHSTRYV (287)
			MM (A1, A3, B50, B57)	302	
			SB(A2, B35, B57)	644	DDYSNTHSTRYV (497)
	LDEGIMVVYKRNIVAHTFKV	0B3	SC(A1, B8)	40	

Table 5 (cont)

Antigen	Peptide Sequence	Peptide ID	Donor and HLA Type	Elispot Results (with 20mer)	Minimal sequence defined (CTLp with ELISPOT)
gH	RQTEKHELLVLVKKAQLNRH	2H4	RK (A23, A24, B27, B41)	313	HELLVLVKKAQL (245) QTEKHELLVLVK (80)
	ALTVSEHVSYVVTNQYLIKG	3B11	MB(A1, A24, B7, B58)	23	LTVSEHVSYVVT (35)
IE-1	CSPDEIMAYAQKIFKILDEE	3F3	CS(A3, A23, B35, B44)	1532	
	EEAIVAYTLATAGVSSSDSL	3G5	MW(A1, A3, B8, B35)	100	
	SEPVSEIEEVAPEEEEDGAE	3G12	SE(A2, A29, B44, B60)	200	
	VLCCYVLEETSVMLAKRPLI	3F10	SB(A2, B35, B57)	2171	
IE-2	TAAKAYAVGQFEQPTETPPE	8562-38	RK (A23, A24, B27, B41)	859	FEQPTETPP (605)
			SB (A2, B35, B57)	326	
	FEQPTETPPEDLDTLSLAIE	8562-39	RK (A23, A24, B27, B41)	791	FEQPTETPP (605)
	MLPLIKQEDIKPEPDFTIQY	8562-1	SB (A2, B36, B57)	132	
	THQLCPRSSDYRNMIIHAAT	8562-32	SC(A1, B8)	173	
	YRNMIIHAATPVDLLGALNL	8562-33	SC(A1, B8)	229	

Table 5 (cont)

Antigen	Peptide Sequence	Peptide ID	Donor and HLA Type	Elispot Results (with 20mer)	Minimal sequence defined (CTLp with ELISPOT)
IE-2	TGPRKKKSKRISELDNEKVR	8562-16	SB(A2, B35, B57)	182	
			SE(A2, A29, B44, B60)	140	
	PVDLLGALNLCLPLMQKFPK	8562-34	SC(A1, B8)	111	
	IQIIYTRNHEVKSEVDAVRC	8562-25	JD(A21, A33, B8, B35)	195	
			SE(A2, A29, B44, B60)	300	
			SB(A2, B35, B57)	303	
	VKSEVDAVRCRLGTMCNLAL	8562-26	SE(A2, A29, B44, B60)	253	
			SB(A2, B35, B57)	320	
pp150	WPRERAWALKNPHLAYNPFR	2B9	RE (A11, A24, B35, B60)	83	ERAWALKNPHLA (546)
	QQLKAWEERQQNLQQRQQQP	1H8	SB(A2, B35, B57)	111	
	SRDAADEVWALRDQTAESPV	2C2	SB(A2, B35, B57)	161	
	VKPLDLNLDRHANTALVNAV	1F11	MW(A1, A3, B8, B35)	70	
	STSQKPVLGKRVATPHASAR	2C10	MW(A1, A3, B8, B35)	62	
	HANTALVNAVNKLVYTGRLI	1F12	MW(A1, A3, B8, B35)	85	

Table 5 (cont)

Antigen	Peptide Sequence	Peptide ID	Donor and HLA Type	Elispot Results (with 20mer)	Minimal sequence defined (CTLp with ELISPOT)
pp65	LNIPSINVHHYPSAAERKHR	1A9	RE (A11, A24, B35, B60)	285	IPSINVHHY (541) HLA B35 published
	ATVQGQNLKYQEFFWDANDI	1D11	CS (A3, A23, B35, B44)	226	QEFFWDANDIY
			RE(A11, A24, B35, B60)	25	QEFFWDANDI (129)
			PP (A1, A24, B8, B14)	202	
			SC (A1 B8)	687	QEFFWDANDI (247)
			MW (A1, A3, B8, B35)	62	
	QEFFWDANDIYRIFAELEGV	1D12	RE(A11, A24, B35, B60)	30	
			PP (A1, A24, B8, B14)	295	
			SC (A1 B8)	704	
			CS (A3, A23, B35, B44)	107	
			MW (A1, A3, B8, B35)	69	
	PQYSEHPTFTSQYRIQGKLE	1C9	RK (A23, A24, B27, B41)	1393	FTSQYRIQGKL (A24 published)
			SE(A2, A29, B44, B60)	546	
			MB(A1, A24, B7, B58)	413	YSEHPTFTSQY (A1 published)

Table 5 (cont)

Antigen	Peptide Sequence	Peptide ID	Donor and HLA Type	Elispot Results (with 20mer)	Minimal sequence defined (CTLp with ELISPOT)
pp65	SQYRIQGKLEYRHTWDRHDE	1C10	SE(A2, A29, B44, B60)	1212	
	VFTWPPWQAGILARNLVPMV	1D9	SE(A2, A29, B44, B60)	533	
			MB(A1, A24, B7, B58)	420	
	ILARNLVPMVATVQGQNLKY	1D10	MB(A1, A24, B7, B58)	500	
			SE(A2,A29, B44, B60)		
			SB(A2, B35, B57)	910	
			MW(A1, A3, B8, B58)	339	
	DQYVKVYLESFCEDVPSGKL	1B7	SE(A2, A29, B44, B60)	127	
	YPSAAERKHRHLPVADAVIH	1A10	SE(A2, A29, B44, B60)	353	
	QYDPVAALFFFDIDLLLQRG	1C7	SB(A2, B35, B57)	411	
	IIKPGKISHIMLDVAFTSHE	1C1	MB(A1, A24, B7, B58)	18	
			SC(A1, B8)	691	
			PP(A1, A24, B8, B14)	177	
			MW(A1, A3, B8, B35)	84	
			SB(A2, B35, B57)	172	
	AHELVCSMENTRATKMQVIG	1B5	MM (A1, A3, B50, B57)	169	
	TRATKMQVIGDQYVKVYLES	1B6	MM(A1, A3, B50, B57)	139	

Table 5 (cont)

Antigen	Peptide Sequence	Peptide ID	Donor and HLA Type	Elispot Results (with 20mer)	Minimal sequence defined (CTLp with ELISPOT)
pp65	MNGQQIFLEVQAIRETVELR	1C5	MM(A1, A3, B50, B57)	277	
			JD(A21, A33, B35, B58)	2985	
	QAIRETVELRQYDPVAALFF	1C6	MM(A1, A3, B50, B57	102	
			SB(A2, B35, B57)	561	
			JD(A21, A33, B35, B58)	2593	
	LTVSGLAWTRQQNQWKEPDV	1B1	MW(A1, A3, B8, B35)	193	
			SC(A1, B8)	695	
	WQPAAQPKRRRHRQDALPGP	1E2	MW(A1, A3, B8, B35)	292	
			JD(A21, A33, B35, B58)	250	
	YRHTWDRHDEGAAQGDDDVW	1C11	SC(A1, B8)	615	
			SC(A1, B8)	295	
			MW(A1, A3, B8, B35)	131	
	TSAGRKRKSASSATACTSGV	1D4	SC(A1, B8)	138	
			JD(A21, A33, B35, B58)	1731	

Table 5 (cont)

Antigen	Peptide Sequence	Peptide ID	Donor and HLA Type	Elispot Results (with 20mer)	Minimal sequence defined (CTLp with ELISPOT)
pp65	HRQDALPGPCIASTPKKHRG	1E4	SC(A1, B8)	109	
	YYTSAFVFPTKDVALRHVVC	IB3	JD(A21, A33, B8, B35)	358	FPTKDVAL (HLA B35) published
	VTTERKTPRVTGGGAMAGAS	1D2	JD(A21, A33, B8, B35)	319	
	QPFMRPHERNGFTVLCPKNM	1B11	SE(A2, A29, B44, B60)	260	
	SICPSQEPMSIYVYALPLKM	1A7	SB(A2, B35, B57)	1770	
	IYVYALPLKMLNIPSINVHH	1A8	SB(A2, B35, B57)	139	
	YYTSAFVFPTKDVALRHVVC	1B3	SB(A2, B35, B57)	1326	

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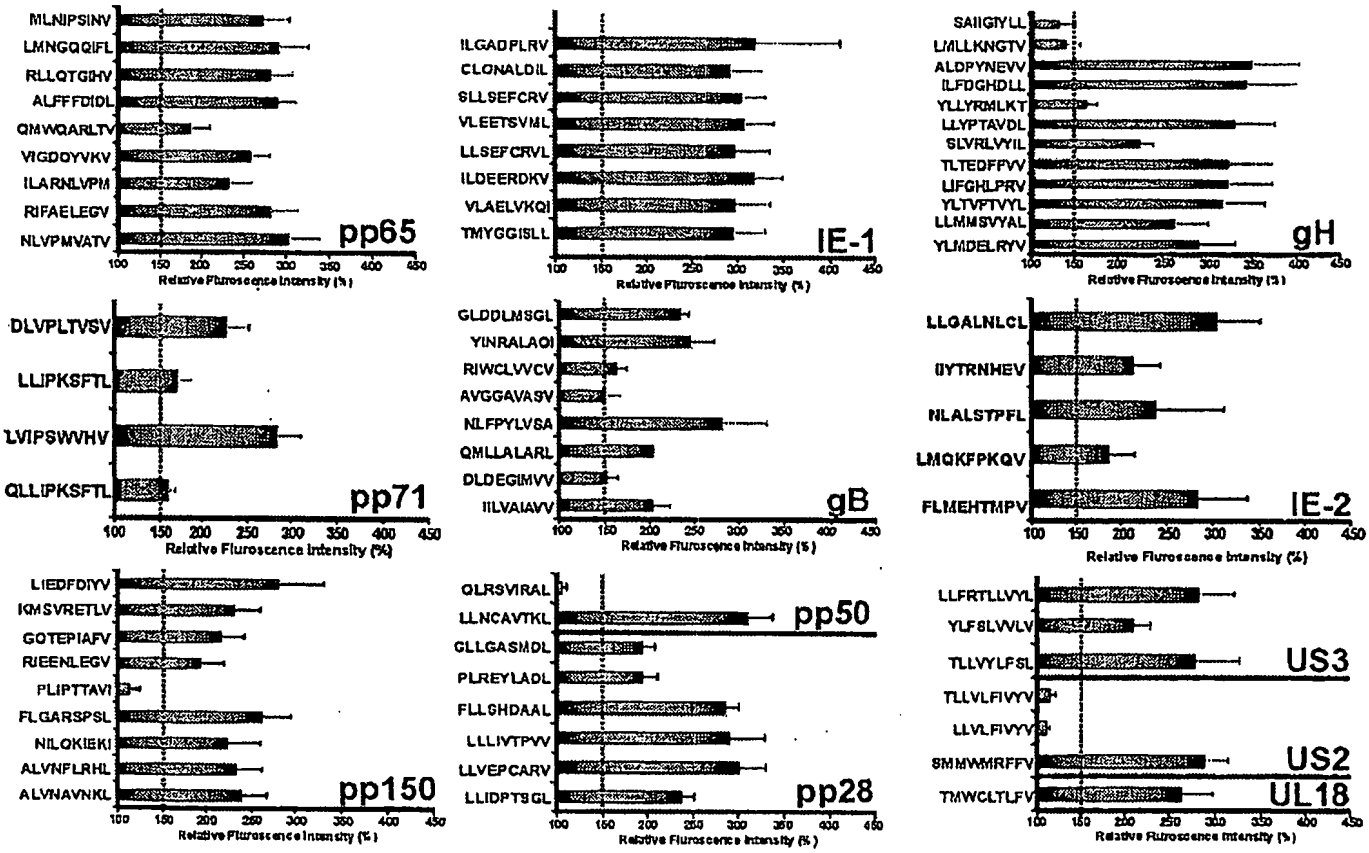

Fig. 1

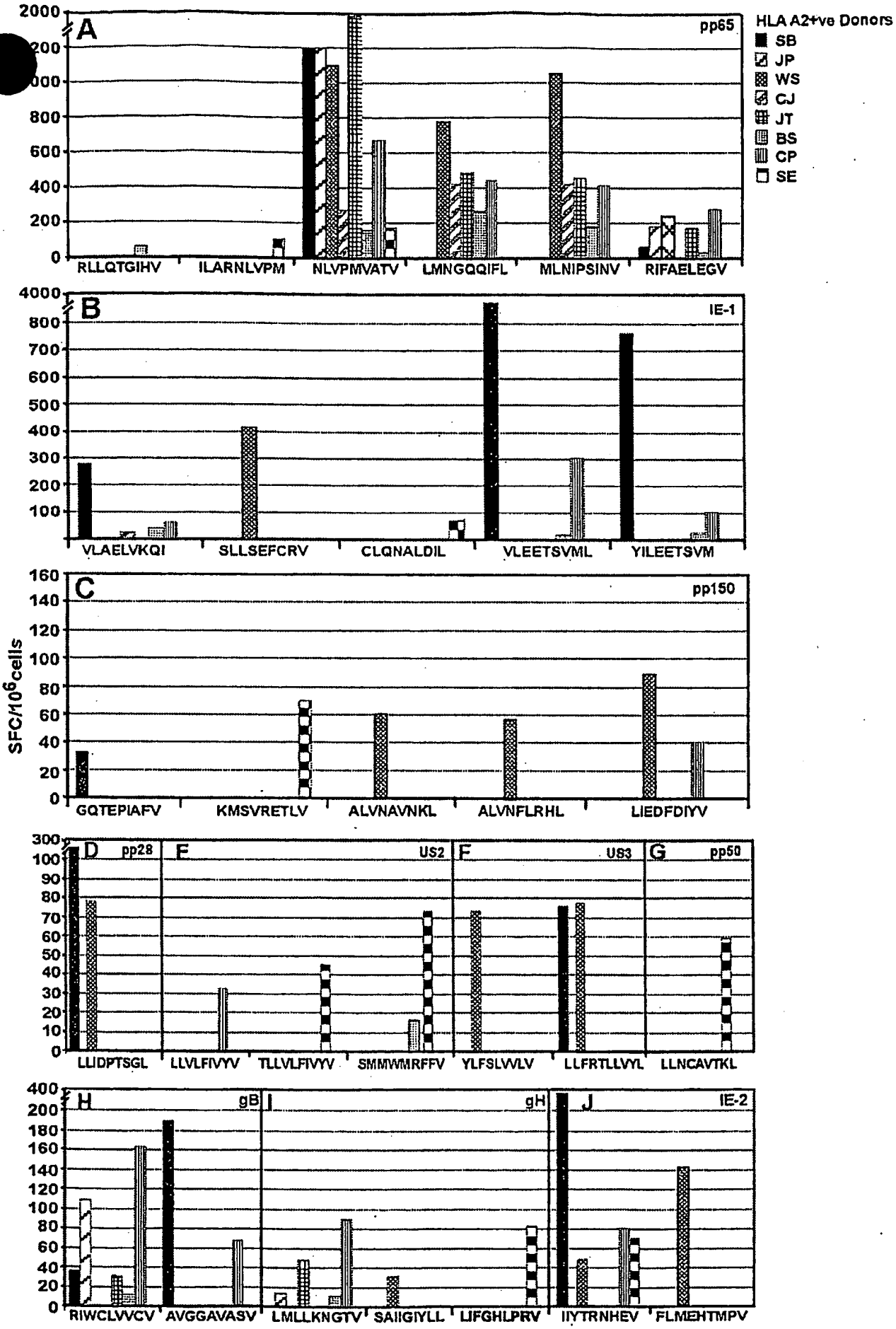

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Fig. 2

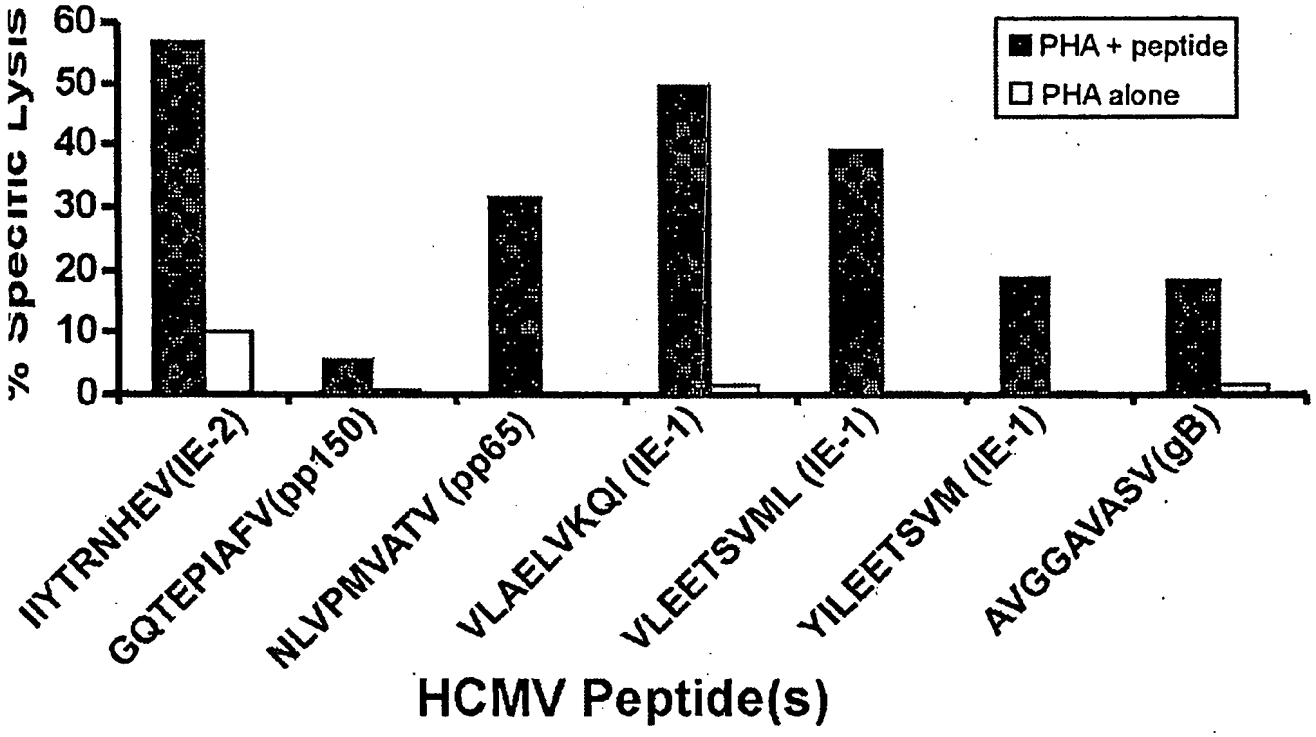
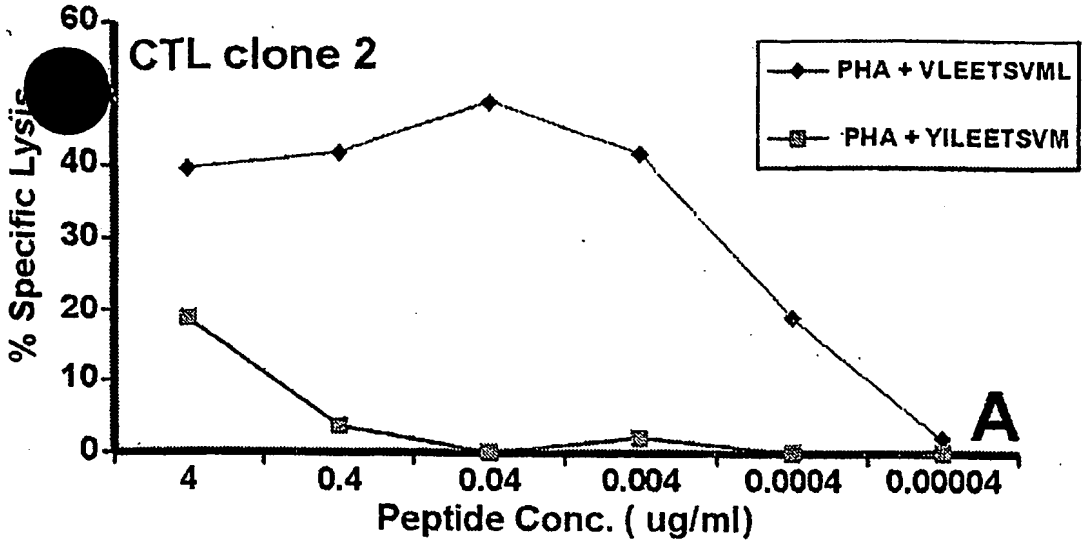
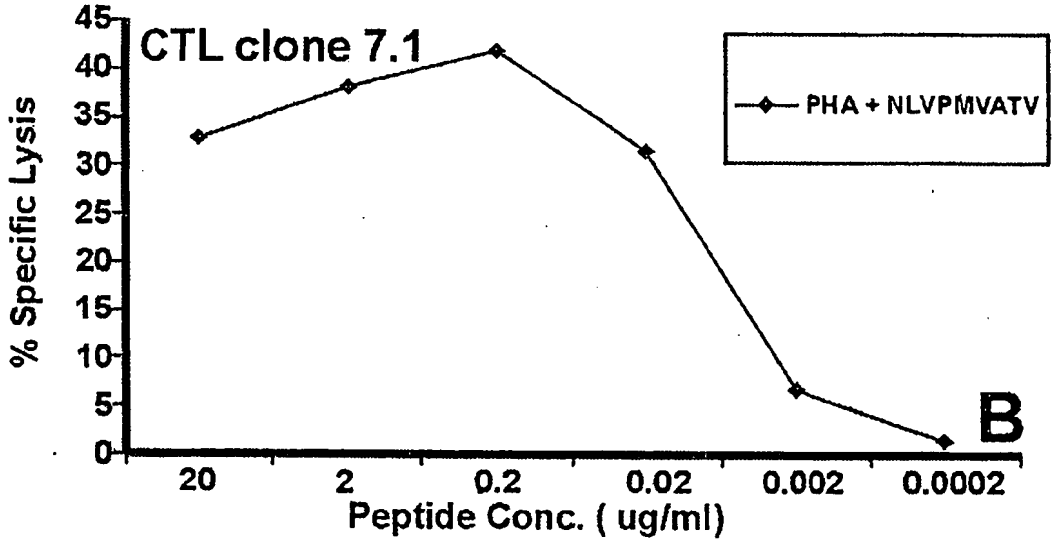


Fig. 3





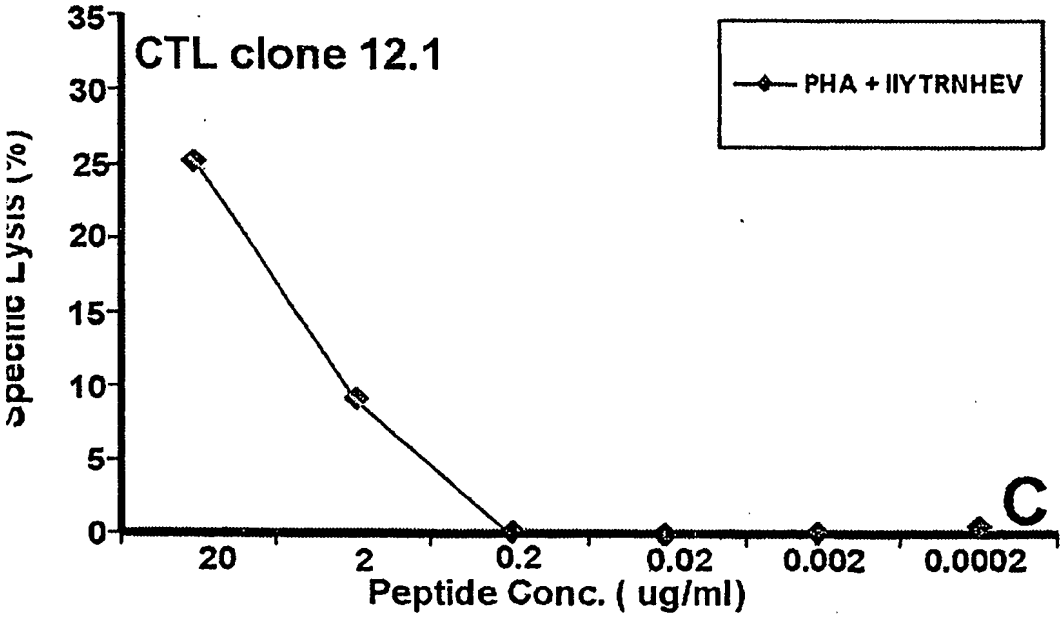


Fig. 4

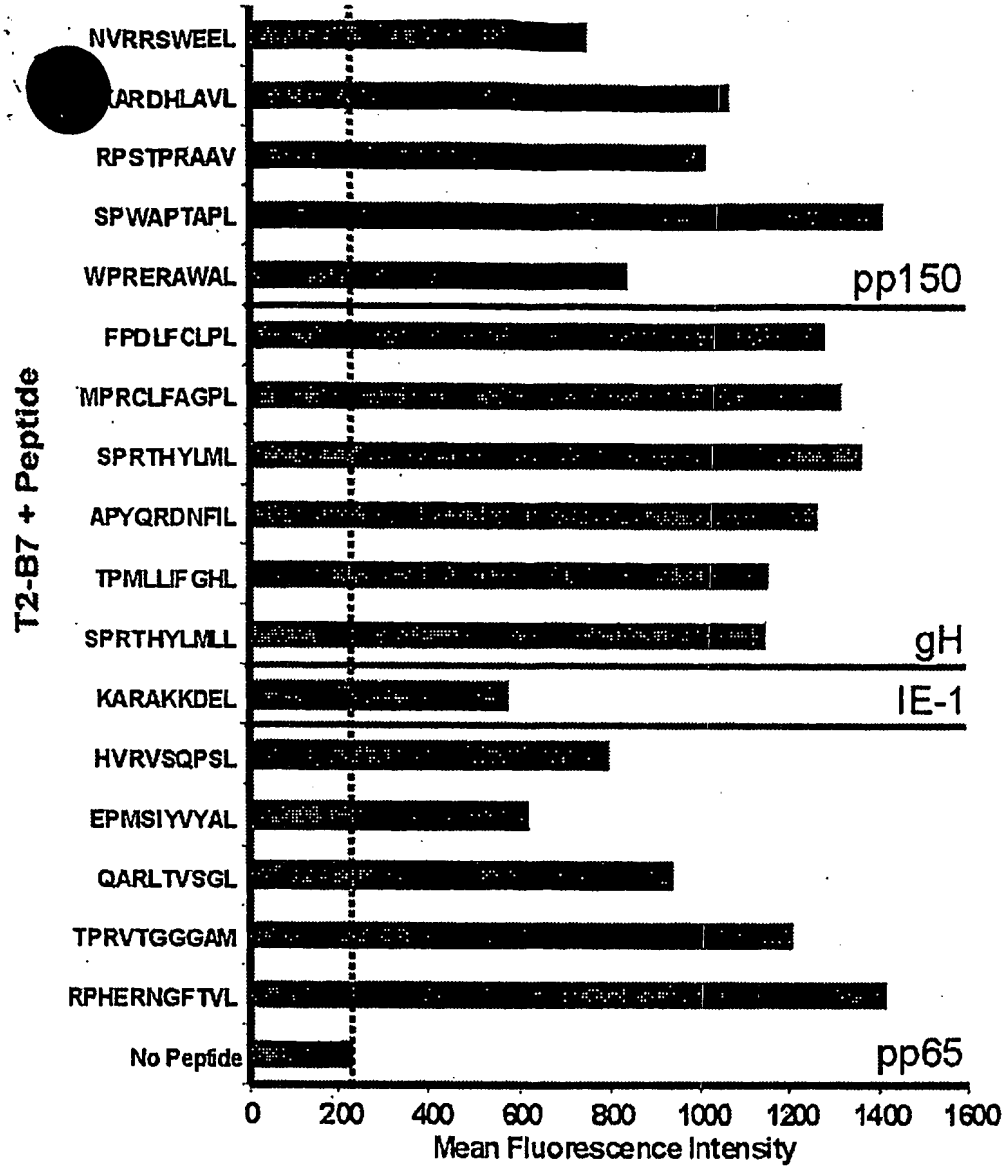


Fig. 5

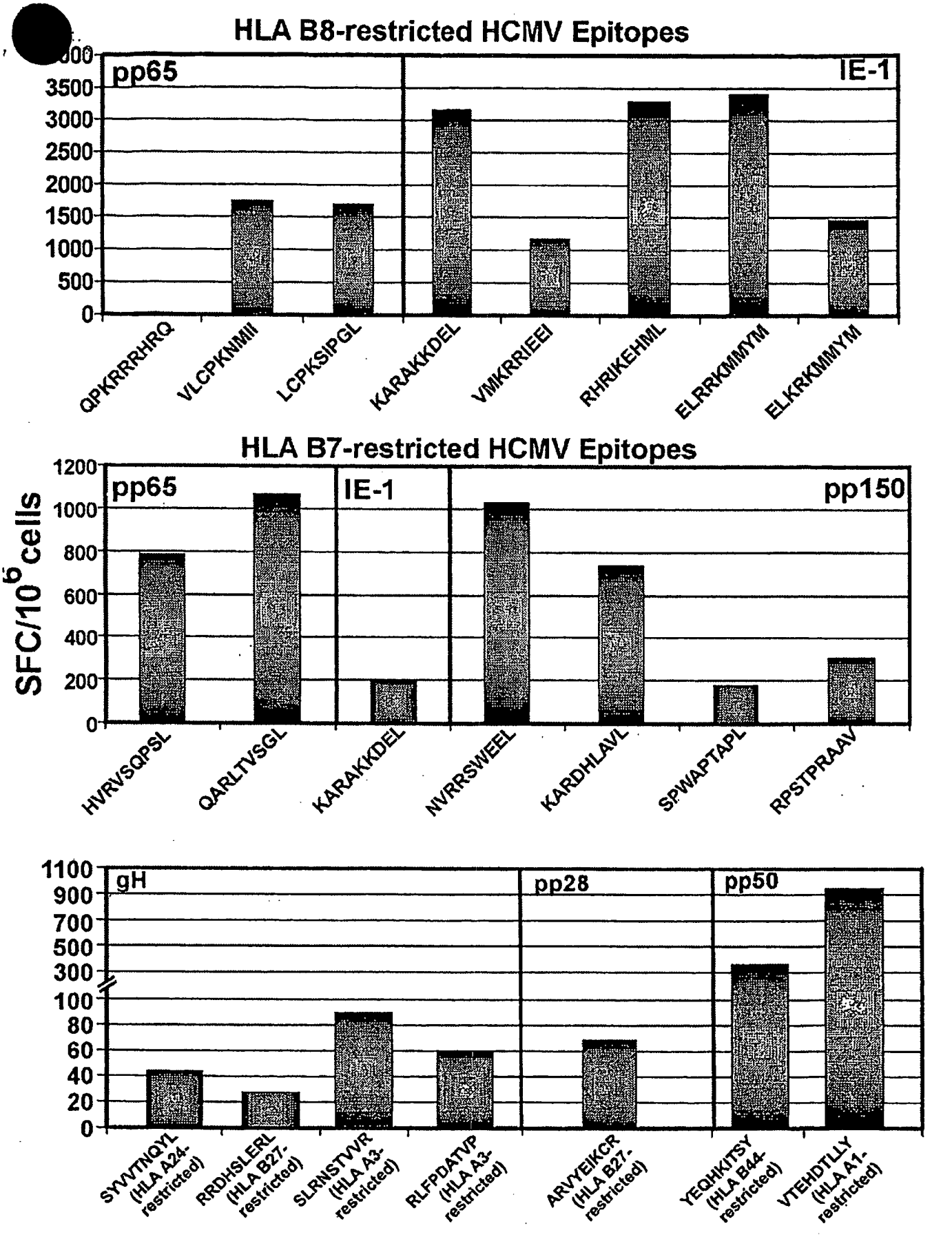


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Fig. 6